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Seroprevalence of Hepatitis E Virus Antibodies (IgG) in the Community of Rawalpindi

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Abstract: Knowledge regarding the prevalence of the hepatitis E virus (HEV) in the general population can indicate public health and personal hygiene practices in a community. HEV spreads through the fecal-oral route and contaminates drinking water through sewage. Moreover, poverty also contributes to its prevalence in developing countries, including Pakistan. A cross-sectional study was conducted on 650 blood samples taken from suspected patients of HEV in the Rawalpindi cantonment area (Pakistan) from April to November 2019 at the Department of Virology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan. Out of them, 444 (68.15%) were male and 206 (31.85%) were female; the detection of anti-HEV IgG antibodies was carried out using a commercial Anti-Hepatitis E virus antibody (IgG) ELISA Kit. The overall anti-HEV IgG prevalence percentages were 19.23% and 4.77% in males and females, respectively. Patients were categorized into eight groups with ages ranging between 1 and 90 years. HEV IgG seroprevalence was the highest in ages 31–40 (6.46%). The study concluded that males aged 40 or above were susceptible and infected with hepatitis E.

Keywords: hepatitis E virus (HEV); IgG seroprevalence; ELISA; AFIP

Citation: Farooqi, M.A.; Ahsan, A.; Yousuf, S.; Shakoor, N.; Farooqi, H.M.U. Seroprevalence of Hepatitis E Virus Antibodies (IgG) in the Community of Rawalpindi. *Livers* **2022**, 2, 108–115. https://doi.org/10.3390/livers2030009

Academic Editor: Giuseppe Colucci

Received: 31 May 2022
Accepted: 25 July 2022
Published: 27 July 2022

1. Introduction

Hepatitis E virus (HEV) is a quasi-enveloped, single-stranded RNA virus of 27–34 nanometers, discovered in 1983. Hepatitis E virus is a significant etiology of viral hepatitis transmitted by the fecal-oral route [1]. The infection with HEV induces acute or subclinical liver diseases like hepatitis A. The HEV infections are endemic in developing countries and spread sporadically with or without a travel history to an endemic area, causing liver infection in the patient [2–4]. Additionally, it is a common cause of water-borne hepatitis in continents like Africa, South America, and Asia (India, Pakistan); however, it is uncommon in developed countries like the United States. An estimated 20 million infections of HEV occur in developing countries, with 3.3 million symptomatic cases and 70,000 deaths [5,6]. The study by Nan et al. (2014) reported a 0.5–3% HEV infection case-fatality rate in developing countries, while the overall case-fatality rate is much higher (15–25%) among pregnant women. The HEV is more prevalent in children between 3 and 18 years of low socioeconomic backgrounds than the respective counterparts in children of higher socioeconomic backgrounds, with 50% to 90% prevalence [7].

This disease resembles hepatitis A infection, with a higher mortality rate in pregnant women. The incubation period of HEV ranges from three to nine weeks and, on average, 40 days. It exhibits a brief prodromal phase of anorexia, nausea, vomiting, and abdominal pain, followed by jaundice. Hepatitis E infection is usually benign and self-limiting in nature [6,8]. However, viral hepatitis may develop into a chronic disease with rapidly progressive cirrhosis in organ transplant recipients, patients with hematological malignancy requiring chemotherapy, and individuals with human immunodeficiency virus (HIV). Hepatitis E virus passes into the sewage, and wherever there are chances of contamination of potable water with sewage containing HEV, outbreaks of HEV infection are likely to occur [9]. The highest infection rate is seen in young adults (15–39 years of age), equally affecting both sexes. The infection is usually mild and self-limiting, without any long-term sequel. However, if it affects females during the third trimester of pregnancy, it can lead to high maternal mortality and frequent fetal loss [10,11]. This disease has produced catastrophic effects on pregnant women, resulting in maternal mortality rates ranging between 20 and 29.3% and prenatal mortality rates of 30.3 per 1000 live births [12].

According to the Pakistan Bureau of Statistics, Pakistan's average monthly wages are 24,080 rupees (105.72 USD) per capita [13]. The low income corresponds to a lack of resources for the resident population, such as clean filtered water and standard hygienic conditions. As hepatitis E virus spreads primarily through the fecal-oral route, poor hand hygiene practices, a lack of education, the unavailability of drinking filtered or boiled water, intermixing sewerage pipelines with drinking water, and terminal inaccessibility to cost-effective water filtration systems lead to the recirculation of the virus in the community. The study by Butt et al. (2016) reported that the serum of Pakistan's 14–26% healthy-appearing population was found to be reactive to anti-HEV IgG [14]. The data regarding the HEV burden in the Rawalpindi community were deficient. Thus, the current study aimed to analyze the frequency of anti-HEV IgG antibodies in the neighborhood of Rawalpindi.

2. Materials and Methods

2.1. Study Area

The study area is district Rawalpindi located within the territory of Punjab province, Pakistan, with a population density of 396.94 persons/sq.km^2, and the population is 51.6% male and 48.41% female. A cross-sectional descriptive study utilizing the consecutive sampling technique was conducted at the Department of Virology at the Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, from March 2019 to November 2019. The blood samples were collected from suspected patients of viral hepatitis infection visiting the outpatient department of the institute.

2.2. Study Population

This study collected 650 blood samples from random patients suspected of hepatitis E virus infection from the Rawalpindi community. The patient's ages ranged from 1 year to 90 years, and a single blood sample for every patient was considered. On average, the suspected patients presented the signs and symptoms of myalgia, arthralgia, anorexia, weight loss, severe dehydration, right upper quadrant pain, diarrhea, fever, fatigue, and loss of appetite. The serum was separated from the whole blood through the serum separator tube upon centrifugation at 2000× *g* for 15–20 min and stored in the refrigerator, with temperatures ranging between 2 and 8 °C. All the incoming samples were analyzed daily, and results were recorded accordingly. The inclusion criteria for the current study included all the individuals who visited the institute's outpatient department and were suspected of harboring HEV.

However, none of the samples were excluded from the study. The samples were divided into eight age groups and categorized according to sex, age, education, employment status, dependency index, source of drinking water, and household occupancy. The frequency of samples received in all age groups was as follows: 1–10 years (n = 69), 11–20 years (n = 113), 21–30 years (n = 118), 31–40 years (n = 97), 41–50 years (n = 62), 51–60 years

(*n* = 57), 61–70 years (*n* = 92), and 71–75 years (*n* = 42). The seroprevalence was estimated in each of the groups. Family dependence means that the study individuals depended on other family members for food, shelter, and living expenses and vice versa for family independence. However, the information was unavailable for some individuals in the employment status category, so we excluded that information in the data analysis.

2.3. Serological Assays

Serum samples were screened for HEV-specific IgG antibodies using colorimetric Enzyme-Linked Immunosorbent Assays Catalogue No. LS-F10219 LifeSpan Biosciences, Inc., Seattle, Washington, DC, USA. HEV-IgG ELISA employs a solid-phase direct ELISA method to detect IgG antibodies to HEV (anti-HEV). Polystyrene 96-well microwell strips were precoated with HEV antigen. A total of 100 µL of serum was added and allowed us to incubate the samples for 45 min in an incubator at 37 °C. The wells were washed with 200 µL of wash buffer to remove unbound serum proteins. Then rabbit antihuman IgG antibodies (anti-IgG) were conjugated using 100 µL horseradish peroxidase, and the unbound enzyme conjugate was removed. An amount of 50 µL of tetramethylbenzidine (TMB) and 50 µL of urea peroxide substrate were added to hydrolyze the bound enzyme conjugate and form a blue-colored product. A total of 50 µL of sulfuric acid was added as a stop solution that turned the solution to a yellow color [15,16]. The amount of color intensity was measured at 450 nm using Thermo Fisher Scientific™ Multiskan™ FC Microplate Photometer, USA, Catalog number: 51119100. Since the objective of the current study was to determine the burden of anti-HEV IgG-reactive patients in the Rawalpindi population, we did not perform ELISA testing on anti-HEV IgM antibodies, and only ELISA testing on anti-HEV IgG antibodies was considered. However, the limitation due to financial resources for the anti-HEV IgM testing was also accounted.

2.4. Ethical Considerations

Ethical approval was taken from the Institutional Ethical Committee, Armed Forces Institute of Pathology (AFIP), Rawalpindi. The study was conducted with relevant laws, institutional guidelines, and ethical standards.

3. Results

Out of the total samples received, only 24% (*n* = 156) were anti-HEV IgG positive, and the rest of the 494 samples were negative for anti-HEV IgG antibodies.

3.1. Prevalence of Anti-HEV IgG Antibodies in Genders

Out of the total samples, 68.31% (*n* = 444) patients were male and 31.69% (*n* = 206) were female. The prevalence of anti-HEV IgG was significantly higher in men than women, i.e., 19.23%. However, the prevalence percentage of women was 4.77% (Table 1).

3.2. Prevalence of Anti-HEV IgG in Age Groups

All the age groups exhibited similar prevalence percentages of the disease burden. Patients' ages were categorized into eight groups with an interval of 10 years. The patients aged <10 years were 69 in number (10.62%), and the percentages of all the subgroups were 17.38% (*n* = 113) for 11–20 years, 18.15 (*n* = 118) for 21–30 years, 14.92% (*n* = 97) for 31–40 years, 9.54% (*n* = 62) for 41–50 years, 8.77% (*n* = 57) for 51–60 years, 14.15% (*n* = 92) for 61–70 years, and 6.46% (*n* = 42) for above 70 years. The study shows that the anti-HEV antibody prevalence percentage in age group less than 10 was 1.08%, age group 11–20 was 3.23%, age group 21–30 was 4.92%, age group 31–40 was 6.46%, age group 41–50 was 2.31%, age group 51–60 was 1.23%, age group 61–70 was 2.77%, and age group above 70 was 2% (Table 1).

Table 1. Prevalence of anti-HEV IgG antibodies according to individual and household-level risk factors.

Variable	No. of Subjects	Prevalence Percentage of Anti-HEV IgG
Age (years)		
Less than 10	69	1.08
10–20	113	3.23
21–30	118	4.92
31–40	97	6.46
41–50	62	2.31
51–60	57	1.23
61–70	92	2.77
Above 70	42	2.00
Sex		
Male	444	19.23
Female	206	4.77
History of Viral Hepatitis		
No	552	20.31
Yes	98	3.69
Household Education		
Primary	172	5.08
Middle	164	4.92
High school	23	0.31
Graduated	291	13.69
Employment Status		
Employed	253	12.62
Housewife	89	2.31
Retired	88	3.69
Dependency Index		
Family-dependent	356	11.85
Non-family-dependent	294	12.15
Drinking Water Filtered		
No	204	19.38
Yes	446	4.62
Housing Occupancy		
1–5	85	1.85
6–11	565	22.15

3.3. History of Hepatitis B and Hepatitis C Infection

Overall, the samples were divided into two groups: having or not having a previous history of hepatitis B or hepatitis C infection. A total of 552 samples had no history of illness, and 98 samples had a history of infection. The prevalence percentages of anti-HEV IgG antibodies were 20.31% and 3.69% in patients with no history of hepatitis B or hepatitis C infection and those with a history of hepatitis B or hepatitis C, respectively (Table 1).

3.4. Household Education

The household education level was determined for each anti-HEV IgG-positive individual in a household. The overall samples were divided into four groups: primary education, middle education, higher secondary school education, and graduation. The history of patients concluded that 172 patients had primary education, 164 patients had middle-level education, 23 had high school education, and 291 were graduates. The prevalence percentage of anti-HEV IgG antibodies in primary-educated patients was 5.08% (n = 33), middle-level-educated patients was 4.92% (n = 32), high-school-level-educated patients was 0.31% (n = 2), and graduates was 13.69% (89), respectively.

3.5. Employment Status

The samples were divided into employed, housewives, and retired. The statistics on the employment status of patients showed that 473 were employed, 89 were housewives,

and 88 were retired. The prevalence of anti-HEV IgG antibodies in employed patients was 12.62% (n = 82), in housewife patients was 2.31% (n = 15), and in retired patients was 3.69% (24), respectively.

3.6. Dependency Index

The patients were divided into two groups: family-dependent and non-family-dependent. The historical analysis of the patients showed that 356 patients were dependent on family, and 294 patients were not dependent on family. The prevalence of anti-HEV IgG antibodies in family-dependent patients was 11.85% (n = 77), while it was 12.15% (n = 79) in non-family-dependent patients.

3.7. Source of Drinking Water

All the patients were divided into two categories having access to filtered drinking water and consuming non-filtered drinking water. A total of 204 patients utilized drinking water that was not filtered and the prevalence of anti-HEV IgG antibodies was 19.38%. While 446 patients used filtered drinking water, the prevalence of anti-HEV IgG antibodies was 4.62%.

3.8. Household Occupancy

The overall samples were divided into two groups. One group comprised patients with 1 to 5 inhabitants in the family, and the second group had 6 to 11 inhabitants. An amount of 85 samples was taken from group one and 565 from group two. The prevalence of anti-HEV IgG antibodies was 1.85% in group one and 22.15% in group two, respectively.

4. Discussion

Viral hepatitis is a common public health concern in third-world countries, especially in the Middle East and Africa. Hepatitis E infection is often associated with outbreaks in low-socioeconomic-status countries such as India, Pakistan, Bangladesh, and Nigeria. Occasionally, sporadic cases have been reported in Europe and South American developed countries [17,18]. The primary route of HEV entry into a human body is the fecal-oral route due to contact with contaminated water in endemic areas; blood-transfusion-based transmission has also been reported according to the high prevalence of anti-HEV IgG among volunteers of blood donation. Several scientific reports have suggested that the consumption of undercooked raw meat accounts for more acute hepatitis than blood transfusion [19,20]. Researchers in Thailand have also reported the association between the hepatitis E virus and close contact with domestic animals. Significant levels of seropositivity were reported in those porcine origin samples [21].

The current study was conducted to ascertain the prevalence of anti-HEV IgG antibodies in patients visiting a tertiary care hospital [19]. The previous studies have reported equal HEV infection rates in both sexes; however, the current study showed a significant difference in prevalence for both genders, where males had 80% positive cases compared to 20% for female patients [22]. This high number of patients in the current study could be associated with fewer female patients registered during the study period. In addition, females in developing countries have fewer treatment opportunities [23]. The prevalence of HEV in the general population varies according to the geographical location of the city or province [24]. Therefore, the HEV prevalence among the general population is reported variably: the tribal people of Egypt (50–100%), the USA (39–42%), Bangladesh (22.5%), and the Netherlands (1.9%) [25–27]. However, in the current study, all the patients registered themselves from the urban area, where the overall positivity rate for anti-HEV IgG was 24%. It has been reported that the severity of anti-HEV IgG antibodies increases with advancing age [28]. The infants are mostly exposed to HEV IgM with less titers of IgG. In a previous study, the HEV seroprevalence percentage increased from 14.3% in people below 31 years to 90.9% in people 61–70 years [29]. Our results showed 1.08% IgG antibody prevalence

against HEV in the patients of less than 10 years; the prevalence was six times higher in the age group 31–40 years, i.e., 6.46%.

Improving personal hygiene and overall public health infrastructure decreases the chances of infections over time. It is consistent with most studies that reported a significant association between age and higher anti-HEV values. Similarly, considerable seroprevalence was found among the adult population older than 60 years in China (70–80%), the population older than 80 years in Bangladesh (67%), and the population aged more than 80 years in Hong Kong (52–60%) [30–32]. The current findings did not endorse the previous literature that stated anti-HEV IgG and IgM antibodies were not associated with gender, race/ethnicity, and HEV seropositivity [33]. The current study showed approximately five times the prevalence of HEV IgG antibodies in males, i.e., 19.23%, compared to the females, 4.77%. The study also highlighted that those patients who had already been infected by hepatitis B or hepatitis C had an almost five times lower prevalence percentage of 3.69% of HEV infection compared to those with a previous history of viral hepatitis with 20.31%. The patients with primary- and middle-level education exhibited similar percentages of HEV infection. However, the graduates were more prone to the disease, and their prevalence was 13.69%. Further, employed patients had a higher prevalence rate, 12.62%, compared to housewives and the retired with an almost similar percentage. The patients dependent on or independent of the family showed an almost identical pattern of the prevalence percentage of anti-HEV IgG antibodies. The use of contaminated drinking water can be associated with HEV infection. This fact can be explained based on statistics of our study that showed that the patients who utilized non-filtered drinking water showed an almost four times higher prevalence rate, i.e., 19.38%, compared with that of filtered water users with 4.62%. Finally, families with more than six inhabitants had a higher prevalence percentage of 22.15% compared to families of five members with a 1.85% prevalence.

Several initiatives could help improve the hygienic conditions of the local population of Rawalpindi. The first and foremost initiative is to promote knowledge on maintaining safe and effective hand hygiene practices in public and private sectors. The state must ensure the authorities provide a cost-effective water filtration system. The boiled or filtered water must be transported in closed containers. The unavailability or low efficiency of municipal waste and sludge removal authorities accounts for the spread of foodborne illness. The public restrooms must be clean and ventilated, and adequate sanitation facilities should be ensured. Social mobilization and behavior change communication initiatives should be started by the government and non-government organizations (NGOs). The unavailability of information regarding the patients' lifestyles was the critical limitation of our study. Moreover, compared to the actual population of Rawalpindi city, the sample size is very small, and the results cannot be generalized throughout the Rawalpindi community.

5. Conclusions

The current study was designed to estimate the prevalence of anti-IgG antibodies in hepatitis E infection suspects. A total of 650 samples were collected and then processed at the Department of Virology, AFIP, Rawalpindi. The study revealed that hepatitis E was more prevalent in males than females in the Rawalpindi community. The prevalence percentages of anti-HEV antibodies in males and females were recorded as 19.23% and 4.77%, respectively. However, adults aged between 31 and 40 were significantly affected by HEV. Their prevalence was 6.46%, higher than that of any other age group. These data show that the 31–40 age group is more likely to have hepatitis E exposure. This infection is also associated with non-filtered water. Almost all the positive results showed a history of drinking non-filtered water. Thus, we can conclude that the origin of infection with hepatitis E virus is contaminated and unfiltered water. The age group from 31 to 40 years mostly is at the workplace and self-employed; that is why water access and hygienic food are not readily available for them compared to early age groups, so they are more susceptible to this viral infection. Moreover, the patients that had used non-filtered water for drinking and had family members over six were more prone to the HEV infection.

Author Contributions: Conceptualization, M.A.F.; methodology, M.A.F. and S.Y.; software, A.A.; validation, N.S. and H.M.U.F.; formal analysis, M.A.F.; investigation, S.Y.; resources, A.A.; data curation, M.A.F.; writing—original draft preparation, M.A.F.; writing—review and editing, H.M.U.F. and A.A.; visualization, A.A.; supervision, A.A.; project administration, N.S.; funding acquisition, H.M.U.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Ethical approval was taken from the Institutional Ethical Committee, Armed Forces Institute of Pathology (AFIP), Rawalpindi. The study was conducted with relevant laws and institutional guidelines and with the ethical standards of Pakistan.

Informed Consent Statement: Written informed consent has been obtained from the patients to publish this paper.

Data Availability Statement: The data supporting this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

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