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Immunological serum markers for hepatitis viral infections: HAV, HBV, HCV to advance of genetic engineering

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Abstract

Viral hepatitis is increasingly present in infections diagnosed around the world. Thousands of individuals are infected by these often-silent diseases that only manifest themselves when clinical signs are very advanced. Hepatitis A virus (HAV) is a vaccine-preventable liver infection. Recombinant vaccines had been applied with the advance of genetic engineering for HBV. New studies have investigated the improvement of the vaccine model of heterological antigens based on hepatitis B virus envelope protein containing HCV antigen as a chimeric vaccine. The aim of this study was to evaluate the detection rates of these viruses that induce liver damage and can progress to cancer depending on the associated epigenetic factors. Thus, between January 10, 2022, and March 31, 2023, about 2.750 samples were collected from 2.713 patients, of which 38.43% were for HCV and 30.25% for HAV research. For HBV, it had been analyzed about 2.222 samples from 1.660 patients, which females were more prevalent (60.60%) and males (39.39%). In all, eight biomarkers of the HBV were investigated and HBsAg marker was non-reactive in 44.01% of the total samples analyzed and 31,41% reactive for anti-HBs. HBV/HCV coinfected patients need more attention. New research about immunological serum markers should be better investigated to correlation the natural history of hepatitis viral infections and hepatocellular carcinoma (HCC) improve tumour genomic and epidemiological surveillance in clinical trials.

Keywords: Hepatitis; Viruses; Viral infections; Serum biomarkers; Recombinant vaccines.

1. Introduction

The laboratory medical corporate organization aims to establish, implement, and maintain the continuous improvement of the management system to ensure better delivery of its products with efficiency and compliance in the provision of proposed services. Strategically meet the needs and expectations of the market to satisfy the target customer and standardize the lab's department through standard operating procedures for greater competitiveness and process optimization. Immunological serum markers to follow the outcome of antiviral therapy as pre-treatment serum CXCL10, metabolic markers and cytokines and chemokines levels associated with hepatocellular injury detected in patients with chronic HBV infection had been investigated with very precision [1]. Several screening tools for the early detection of HCC had been applied for the assessment risk of HCC in viewing a better prognostic for these patients with cirrhosis [1].

New research about immunological serum markers should be better investigated to correlation with the natural history of hepatitis viral infections and improve epidemiological surveillance in clinical trials. In addition, also hepatocellular

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carcinoma (HCC) antiviral therapy reflects the new challenges in the tumor genomic surveillance and predictive genetic factors associated with risks in the development and worsening of liver injuries and damage of this chronic patients.

So, it is of paramount importance the monitoring surveillance markers (hepatitis B core-related antigen (HBcrAg) and Mac-2 binding protein glycan isomer (M2BPGi) and tumors markers (alpha fetoprotein (AFP), protein induced by vitamin K absence or antagonist-II; (PIVKA-II), *Lens culinaris* agglutinin-reactive fraction of alpha-fetoprotein (AFP-L3), Dickkopf-1 (DKK-1) to HBV infection and liver damage progression as chronic hepatitis, cirrhosis, hepatocellular carcinoma (HCC), until recurrent HCC during the years, may be some risks of complications involved in the stages of the natural historical of disease [2].

The National Institutes of Health (NIH) National Cancer Institute (NCI) defines a biomarker as "a biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, condition, or disease (https://www.cancer.gov/publications/dictionaries/cancer-terms/def/biomarker) (Accessed on 09 September 2023) (Hayashi et al., 2021). There is a useful marker for monitoring patients with chronic hepatitis B as serum hepatitis B core-related antigen (HBcrAg) reflects the amount of covalently closed circular DNA (cccDNA) and transcriptional activity in hepatocytes [3]. These chronic patients had been treated with nucleus(t)ide analogs (NAs) include lamivudine (LAM), telbivudine (LdT), tenofovir disoproxil fumarate (TDF), adefovir (ADV), and entecavir (ETV), with ETV and TDF or tenofovir alafenamide fumarate (TAF) [4].

1.1. Hepatitis A virus (HAV)

Hepatitis A virus (HAV) belongs to the *Picornaviridae* family, genus *Hepatovirus*. The occurrence of outbreaks of hepatitis A in recreational water and drinking water have been constantly reported since it is an enteric virus transmitted by the fecal-oral route in which the ingestion of contaminated water and food or even direct contact with people the person are forms of viral infection. Among the classic symptoms, there is jaundice, choluria and fecal acholia [5, 6]. Hepatitis A vaccines are based on classic first-generation inactivated virus vaccines and have been developed by different biopharmaceuticals such as Havrix® (Glaxo Smith Kline Biologicals), Twinrix® also by the same biopharma, but in this case it is a multiple compound vaccine by several immunogens, and the vaccine VAQTA® from the manufacturer Merck & Co. Regarding the same biopharmaceutical, the vaccine against Herpes-zoster (Merck & Co) developed the so-called Zostavax® with attenuated live virus available on the market. Herpes simplex viruses type 1 and 2 belong to risk class 2 of the laboratory biosafety level [5].

Hepatitis A is a vaccine-preventable liver infection caused by the hepatitis A virus (HAV). HAV is found in the stool and blood of people who are infected and Most people with hepatitis A do not have long-lasting illness. Hepatitis A can be transmitted through close personal contact with an infected person or through eating contaminated food or drink [7].

About 10 outbreak-associated cases of hepatitis A reported that frozen organic strawberries are the likely source of this outbreak. The hepatitis A virus strain causing illnesses in this outbreak is genetically identical to the strain that caused a foodborne hepatitis A outbreak in 2022, which was linked to fresh organic strawberries imported. Symptoms of hepatitis A usually appear 2 to 7 weeks after exposure and can include Yellow skin or eyes; Not wanting to eat; Upset stomach; Stomach pain; Throwing up; Fever; Dark urine or light-colored stools; Joint pain; Diarrhea and Feeling tired [7].

1.2. Hepatitis B virus (HBV)

Hepatitis B virus (HBV) is classified in the *Hepadnaviridae* family divided into two genera: *Orthohepadnavirus* and *Avihepadnavirus*. The genome is composed of a 3,200base pairs (bp) of a circular double stranded DNA presenting four open reading frames (ORFs) designated as: Pre–S/S, Pre-C/C, P and X [8]. The Pre–S/S ORF corresponds to the Hepatitis virus surface gene (HBsAg). HBV is a short-term disease and is one of the major causative agents of chronic liver illness. For others, it can become a long-term, chronic infection like liver disease or liver cancer [7].

HBV infection produces two types of viral particles: full, spherical, HBV genome–containing infectious particles (42nm), as well as non–infectious spherical or filamentous (22nm) subviral particles composed exclusively of HBsAg. An expression system (as used in the Papilloma vaccines, the first recombinant vaccine licensed and produced from yeast expression) was used for Hepatitis B surface– antigen isolation from human plasma of chronic HBV patients (Heptavax–B, Merck & Co), and was released in 1981 [5, 8].

There are two types of successful vaccines based on virus–like particles (VLP) involving Hepatitis B virus surface antigen (HBsAg) and core antigen (HBcAg) expressed in *Escherichia coli*. Conditions on the immunogenicity can be tested in mice with alternative routes of administration of HBV vaccine and novel formulations assays. The development

of recombinant vaccines composed exclusively of HBsAg (Engerix–B, SmithKline and Recombivax, Merck & Co) was possible with the advance of genetic engineering. New studies have investigated the improvement of the vaccine model of heterological antigens based on hepatitis B virus envelope protein containing HCV antigen as a chimeric vaccine [5].

Clinicians should screen all adults aged 18 years and older for HBV infection at least once during their lifetime using the triple panel test which includes hepatitis B surface antigen (HBsAg), antibody to hepatitis B surface antigen (anti-HBs), and total antibody to hepatitis B core antigen total (anti-HBc). In the United States, there are three single-antigen hepatitis B vaccines (Engerix-B; Recombivax HB; Heplisav-B); one three-antigen vaccine (PreHevbrio), and three combination vaccines currently licensed (i) Pediarix: Combined hepatitis B, diphtheria, tetanus, acellular pertussis (DTaP), and inactivated poliovirus (IPV) vaccine; (ii) Twinrix: Combined hepatitis A and hepatitis B vaccine; (iii) Vaxelis: Combined DTaP, IPV, *Haemophilus influenzae* type b, and hepatitis B vaccine) [7].

1.3. Hepatitis C virus (HCV)

Hepatitis C virus (HCV) affects more than 70% of an estimated population of 170 million, inducing chronic lesions of hepatitis, severe fibrosis, cirrhosis, and hepatocellular carcinoma. The viral envelope glycoproteins E1 and E2 are the target of neutralizing antibody responses, but they are also the two most variable proteins. In the research by Simões and collaborators on the chimeric development of a vaccine against HCV, however, there are no vaccines available on the market [5]. Detection of the HCV viral genome is done by molecular RT-PCR assay. The therapy adopted is the use of conventional or pegylated interferon alpha (IFN-α) in association with ribavirin. However, the treatment will depend on the virus genotype and the viral load obtained by q-RT-PCR (Real-time PCR) [9,10].

Hepatitis C virus (HCV) belongs to the *Nidovirales* order, *Flaviridae* family, *Hepacivirus* genus. HCV is an enveloped virus presenting a single strand of positive polarized RNA genome with approximately 9.400 nucleotides. HCV illness is a chronic infection that affects more than 2% of the global population and causes end– stage liver diseases as chronic hepatitis. It is a worldwide public health problem that affects more than 70% of the estimated 170 million people inducing chronic lesions hepatitis. This virus leads to severe fibrosis and cirrhosis, hepatic failure, or hepatocellular carcinoma. HCV has a higher rate of mutation existing inside an individual as *quasispecies*. HCV is divided into six genotypes and multiple subtypes. The envelope glycoproteins E1 and E2 are the natural targets to neutralizing antibodies response but are also the two of the most variable HCV proteins. Production of specific antibodies in rabbits against conserved and potentially immunogenic peptides of the HCV envelope glycoprotein E2 has been described. HCV displays a high variability and is classified into seven genetically distinct genotypes which differ by approximately 30% at the nucleotide level. Envelope (E1/E2) proteins of HCV may generate neutralizing antibodies. At the end N–terminus of the E2 protein there is a region of 27 amino acids called hypervariable region 1 (HVR1), very important in neutralizing HCV. Despite the high degree of variability of E2 protein, some amino acid positions are conserved, and this protein is the target of several neutralizing monoclonal antibodies.

2. Material and methods

This is a descriptive epidemiological study, which evaluated cases of viral hepatitis A viral (HAV), hepatitis B viral (HBV) and C (HCV) at Rio de Janeiro city. All samples were collected during the period from January 1, 2022, to March 31, 2023 at the All Lab. Hepatitis B virus research were carried out using the Chemiluminescence method – CLIA. The presence (reactive) or absence of anti-HBV and HCV antibodies (non-reactive) was also investigated by the Electrochemiluminescence method - ECLIA.

3. Results

A total of 2.750 samples were collected from 2.713 patients, of which 38.43% were for HCV and 30.25% for HAV research. On 2022, there was no reactive sample for the biomarker HBc IgM and IgG between the January and December. It had been analyzed about 2.222 samples from 1.660 patients, which females were more prevalent (60.60%) and males (39.39%). In all, eight biomarkers of the hepatitis B virus (HBV) were investigated, of which the HBsAg marker was non-reactive in 44.01% of the total samples analyzed and 31,41% reactive for anti-HBs.

For another hand, in the year 2023, there was no reactive sample for the biomarker HBc IgM and HBsAg between the January and March. In these months, 472 samples had been collected of 411 patients. So, there was a higher prevalence of females (54.98%) and males (45.01%). In all, 8 biomarkers for the hepatitis B virus (HBV) were investigated, of which the research for non-reagent HBs Ag with 47.66% and Anti-HBs with 31.99% stand out in 2023.

The highest percentage of investigated samples (98.38%) was recorded in March 2022 with a average proportion of 55.25 ± 12.96 (CV = 0.234) for the non-reactive IgM biomarker (Table 1 -2). All serological biomarkers of the hepatitis A virus (HAV) are better represented in figure 1.

Table 1 Percentage of HAV biomarkers by months in the 2022 and 2023 year

Biomarkers	**IgM**			**IgG**		
Months/2022	**Reactive (%)**	**Non-Reactive (%)**	**Inconclusive (%**	**Non-Reactive (%)**	**Reactive (%)**	**Inconclusive (%)**
January	0.0	96.55	0.0	0.0	3.44	0.0
February	1.69	89.83	0.0	1.69	6.77	0.0
March	0.0	98.38	0.0	1.61	0.0	0.0
April	2.32	90.69	1.16	1.16	4.65	0.0
May	0.74	52.23	0.74	9.70	36.56	0.0
June	0.0	84.72	1.38	6.94	6.94	0.0
July	0.0	95.08	0.0	1.63	3.27	0.0
August	0.0	95.34	2.32	0.0	0.0	2.32
September	0.0	88.46	0.0	0.0	11.53	0.0
October	0.0	82.35	0.0	11.76	5.88	0.0
November	0.0	98.18	0.0	0.0	1.81	0.0
December	0.0	98.27	0.0	0.0	1.72	0.0
Months/2023	IgM (%)			IgG (%)		
January	0.0	100.0	0.0	0.0	0.0	0.0
February	0.0	100.0	0.0	0.0	0.0	0.0
March	0.0	92.0	0.0	2.0	0.0	0.0

Table 2 Analysis of means, standard deviation, and coefficient of variation of HAV biomarkers in the 2022 and 2023 years

Biomarkers	**IgM**			**IgG**		
Year	**Reactive**	**Non-Reactive**	**Inconclusive**	**Non-Reactive**	**Reactive**	**Inconclusive**
2022	0.33± 0.65 (CV = 1.96)	55.25± 12.96 (CV=0.234)	0.33 ± 0.49 (CV=1.484)	2.16 ± 3.78 (CV = 1.747)	6.33± 13.57 (CV = 2.143)	0.08 ± 0.288 (CV = 3.469)
2023	0.0	52.66 ± 6.11 (CV = 0.116)	0.0	0.33 ± 0.577 (CV = 1.72)	0.0	0.0

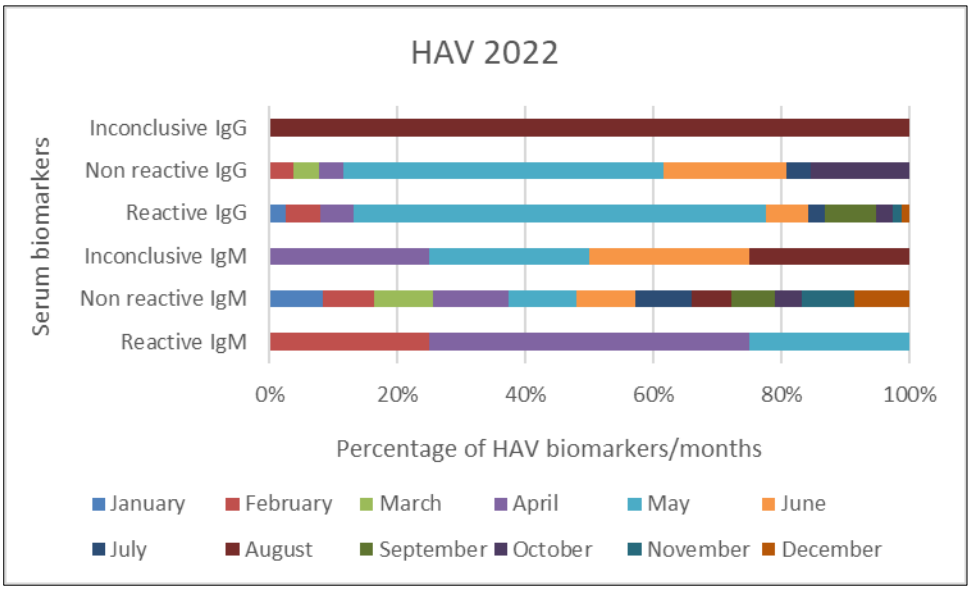

Figure 1 Serum biomarkers of the hepatitis A virus (HAV) investigated in the 2022 year.

About 774 blood samples were analyzed for the hepatitis A virus (HAV) from 740 patients with a average proportion of 61.66 ± 22.08 (CV = 0.358). Thus, 417 were male with a mean ratio of 34.75 ± 8.34 (CV = 0.24) and 323 were female with 26.91 ± 14.46 (CV = 0.537) (Table 3).

Table 3 Percentage of HAV samples collected by gender among months/years.

Gender	**Female**	**Male**
Months/2022	**(%)**	**(%)**
January	43.85	56.14
February	38.59	61.40
March	52.45	47.54
April	47.56	52.43
May	55.37	44.62
June	39.39	60.60
July	37.93	62.06
August	33.33	66.66
September	40.00	60.00
October	38.23	61.76
November	36.36	63.63
December	40.35	59.64
Total	43.64	56.35

In 2023, 162 samples were analyzed, of which 159 patients were analyzed, mostly 62.26% of male samples with a average proportional of 33 ± 8.54 (CV = 0.258) and 37.73% of females with a mean proportion of 20 ± 3.46 (0.173) (Table 4 and Figure 2).

Table 4 Analysis of means, standard deviation and coefficient of variation of all patients HAV including female and male collected in the 2022 and 2023 years.

Gender	Female	Male	Patients
Year			
2022	26.91 ± 14.46 (CV = 0.537)	34.75 ± 8.34 (CV = 0.24)	62.66 ± 22.08 (CV = 0.358)
2023	20 ± 3.46 (CV = 0.173)	33 ± 8.54 (CV = 0.258)	53 ± 5.56 (CV = 0.105)

In 2022 years, all samples collected among female, and male investigated in the serological biomarkers of the hepatitis A virus (HAV) are better represented in figures 2 - 3 showed in 2023 y.

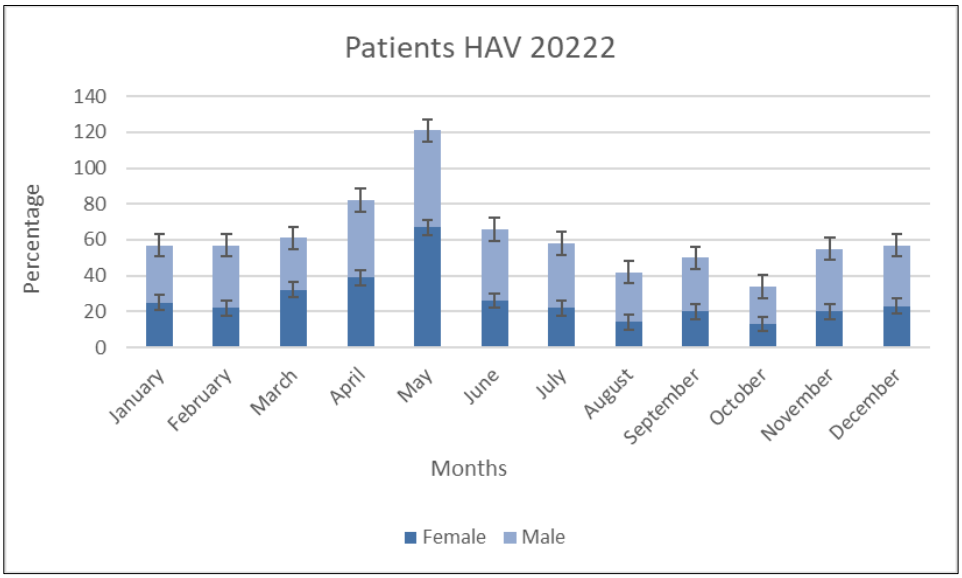

Figure 2 Samples collected among female and male investigated serum biomarkers of the hepatitis A virus (HAV) investigated in the 2022 year.

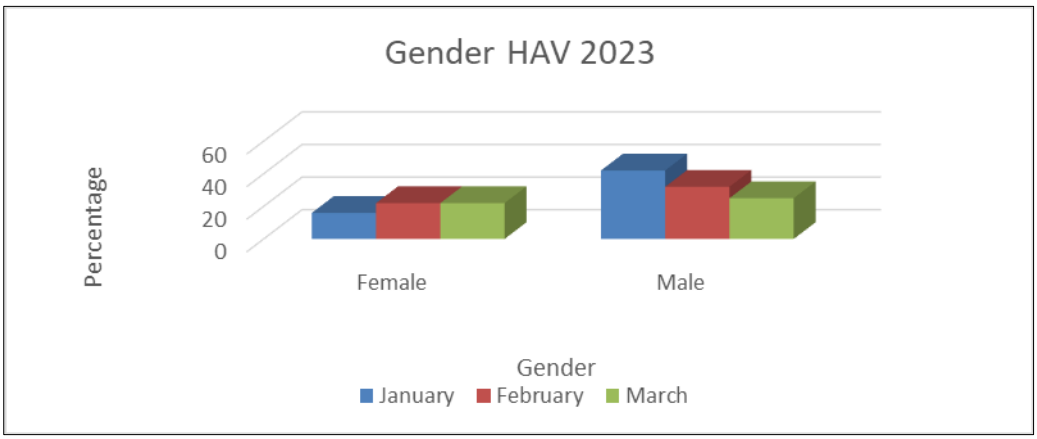

Figure 3 Samples collected among female and male investigated serum biomarkers of HAV in the 2023 year.

About 854 blood samples were collected for HCV virus testing with average proportion of 71.16 ±15.18 (CV = 0.213) of which most samples were taken from 440 (51.52%) men with 36.66 ± 6.87 (CV = 0.187) and 414 (48.47%) women with 34.5 ±10.12 (CV = 0.293), showed by table 5. The anti-HCV biomarker was non-reactive mainly in April 2022 (figure 4).

Table 5 Percentage of samples HCV collected by gender among months/years.

Gender	**Female**	**Male**
Months/Year 2022	**(%)**	**(%)**
January	47 (54,65)	39 (45,34)
February	35 (50,72)	34 (49,27)
March	47 (58,02)	34 (41,97)
April	52 (53.60)	45 (46.39)
May	43 (46.73)	49 (53.26)
June	30 (41.66)	42 (58.33)
July	28 (41.17)	40 (58.82)
August	27 (44.26)	34 (55.73)
September	29 (48.33)	31 (51.66)
October	22 (48.88)	23 (51.11)
November	24 (40.00)	36 (60.00)
December	30 (47.61)	33 (52.38)
Total	414 (48.47)	440 (51.52)
Months/Year 2023		
January	22 (33.33)	44 (66.66)
February	31 (46.26)	36 (53.73)
March	39 (55.71)	31 (44.28)
Total	92 (45.32)	111 (54.67)

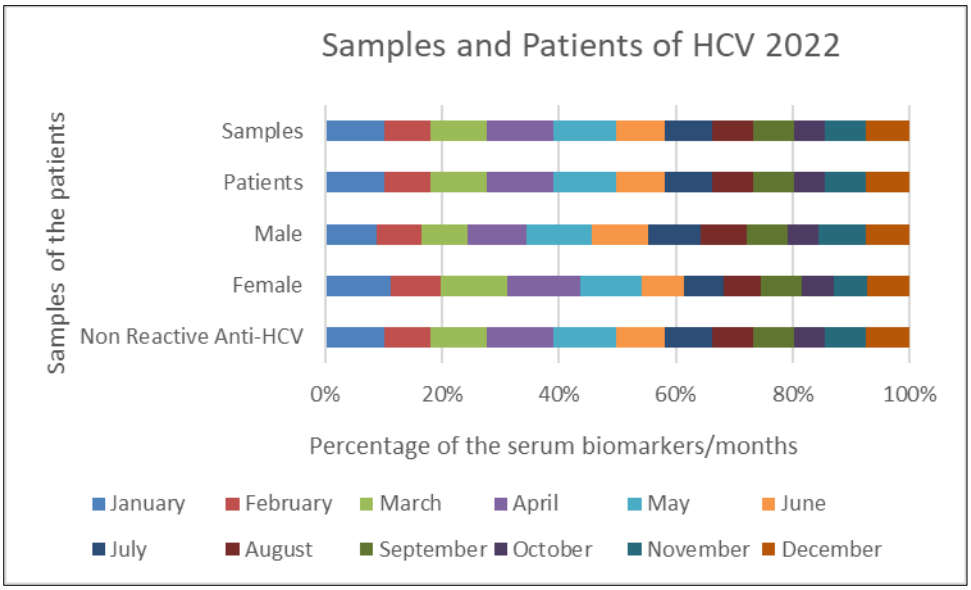

Figure 4 Serum biomarkers of the hepatitis C virus (HCV) investigated in the 2022 year.

In the year 2023, about 203 samples were collected with a prevalence of 54.67% for men with an average proportional of 37± 37.38 (CV = 1.01) and 45.32% for women with an average proportional of 30,66 ± 31.44 (CV = 1.025), represented in the table 6 and figure 5.

Table 6 Analysis of means, standard deviation and coefficient of variation of all patients HCV including female and male collected in the 2022 and 2023 years.

Gender	Female	Male	Patients
Year			
2022	34.5 ± 10.12 (CV = 0.293)	36.66 ± 6.87 (CV = 0.187)	71.1 6± 15.18 (CV = 0.213)
2023	30.66 ± 31.44 (CV = 1.025)	37 ± 37.38 (CV = 1.01)	67.66 ± 67.68 (CV = 1.0)

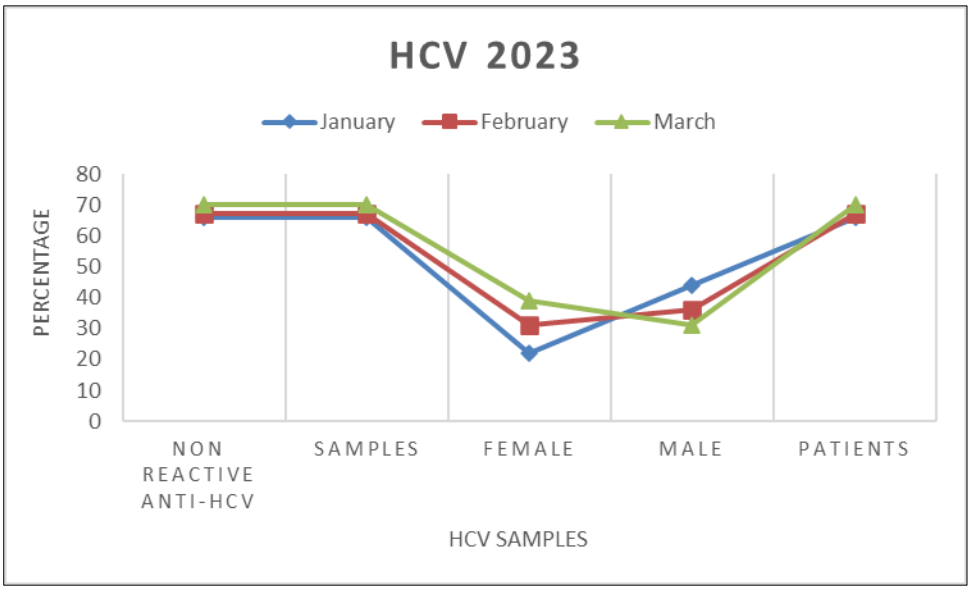

Figure 5 Serum biomarkers of the hepatitis C virus (HCV) investigated in the 2023 year.

Comparing the percentual between January and December months in the 2022, there was no reactive sample for HBc IgM and IgG of the 2.222 samples analyzed, from 1660 patients, of which females were more prevalent with 60.60% and males with 39.39% (Table 7 and Figure 6).

Table 7 Percentage of HBV biomarkers by months in the 2022 year

Biomarkers	HBc IgM	HBc IgG	Anti-HBs		HBsAg		Anti-Hbe	Hbe Ag	Anti-HBc	HBc Ag
Months/2022	Non-Reactive (%)	Non-Reactive (%)	Reactive (%)	Non Reac. (%)	Reactive (%)	Non Reac. (%)	Non-Reactive (%)	Non-Reac. (%)	Non-Reac. (%)	Non-Reac. (%)
January	5.70	5.70	35.30	11.40	0.0	0.92	5.48	5.48	0.0	0.0
February	6.63	6.16	29.38	6.63	0.0	38.86	0.0	0.0	6.16	6.16
March	0.44	5.38	26.00	12.55	0.0	47.98	0.0	3.58	4.03	3.58
April	1.08	3.80	26.08	11.41	0.0	56.52	0.0	0.54	0.54	1.08

May	2.03	2.03	33.50	13.19	0.0	47.20	1.01	0.0	0.0	0.0
June	3.82	3.82	38.25	13.66	0.0	38.25	1.09	0.0	0.0	1.09
July	2.11	2.11	32.39	10.56	0.0	52.81	0.0	0.0	0.0	0.0
August	4.26	4.87	28.65	9.14	0.0	40.85	9.75	2.43	0.0	0.0
September	3.80	5.71	22.85	11.42	0.0	56.19	0.0	0.0	0.0	0.0
October	4.16	4.16	22.91	11.45	0.0	50.0	6.25	1.04	0.0	0.0
November	0.80	0.80	37.09	10.48	0.80	50.0	0.80	0.80	0.0	0.0
December	0.0	0.72	35.03	11.67	1.45	51.09	0.0	0.0	0.0	0.0
Total	3.28	4.14	31.41	11.16	0.13	44.01	2.34	1.80	1.03	1.12

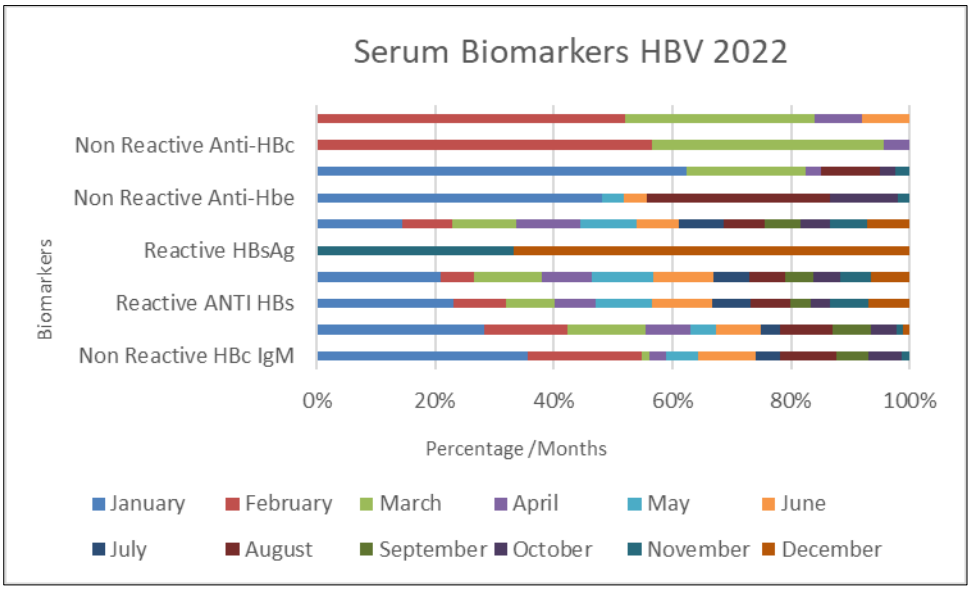

Figure 6 Serum biomarkers of the hepatitis B virus (HBV) investigated in the 2022 year.

In all, 8 biomarkers of the hepatitis B virus (HBV) were investigated, of which the HBsAg was non-reactive or negative in 44.01% of the total samples analyzed and 31.41% reactive for anti-HBs (Table 8-10 and Figure 7).

Table 8 Analysis of means, standard deviation and coefficient of variation of HBV biomarkers in the 2022-2023 years

Biom	**HBc IgM**	**HBc IgG**		**Anti-HBs**		**HBsAg**		**Anti-Hbe**	**Hbe Ag**	**Anti-HBc**	**HBc Ag**
Year	Non-Reactive	Reactive	Non-Reactive	Reactive	Non-Reac.	Reactive	Non-Reac.	Non-Reactive	Non-Reac.	Non-Reac.	Non-Reac.
2022	6.08 ± 7.31 (0.003)	0.0	7.66±6.90 (0.900)	58.16±35.53 (0.610)	20.66± 11.44 (0.553)	0.25±0.62 (2.484)	81.5±25.85 (0.317)	4.33±7.98 (1.841)	3.33±7.22 (2.168)	1.91±4.33 (2.263)	2.083±4.144 (1.989)
2023	2.00±00 (0.00)	0.66±1.15 (1.732)	1.33±1.15 (0.865)	75.5±17.61 (0.23)	25.00±6.55 (0.262)	0.0	75.00±13.07 (0.174)	0.33±0.577 (1.732)	0.33±0.577 (1.732)	1.00±1.00 (1.00)	1.00±1.00 (1.00)

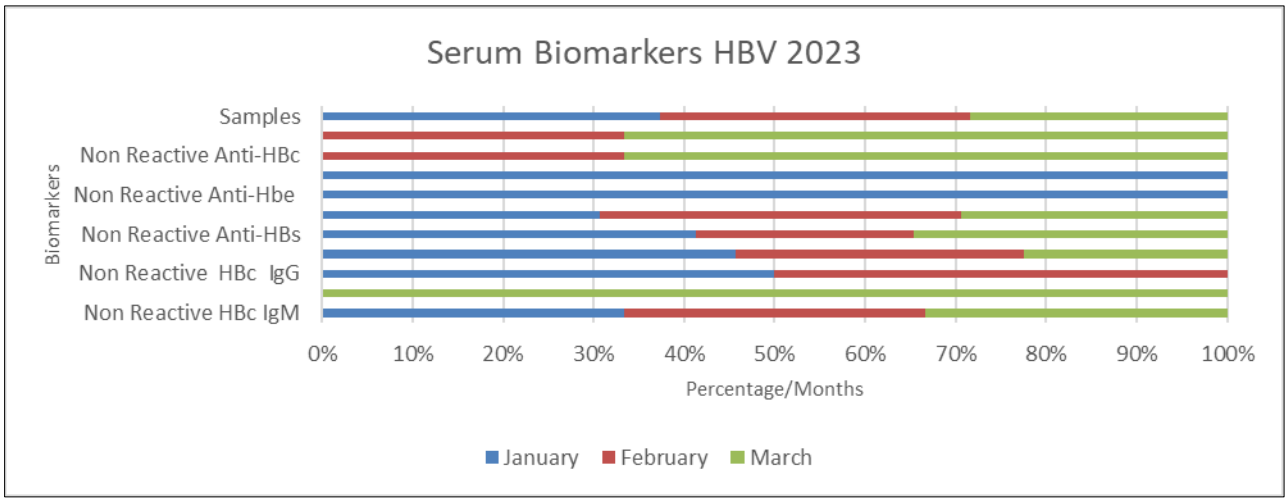

Figure 7 Serum biomarkers of the hepatitis B virus (HBV) investigated in the 2023 year.

Table 9 Percentage of samples HBV collected by gender among months/years.

Gender	**Female**	**Male**
Months/Year 2022	**(%)**	**(%)**
January	70.39	29.60
February	56.61	43.38
March	61.81	38.18
April	62.25	37.74
May	63.42	36.57
June	62.25	37.74
July	59.63	40.36
August	51.88	48.11
September	57.64	42.35
October	56.06	43.93
November	54.78	45.21
December	51.61	48.38
Total	60.60	39.39
Months/Year 2023		
January	50.29	49.70
February	52.27	47.72
March	65.17	34.82
Total	54.98	45.01

Table 10 Percentage of HBV biomarkers by months in the 2023 year

Biomarkers	HBc IgM	HBc IgG		Anti-HBs		HBsAg	Anti-Hbe	Hbe Ag	Anti-HBc	HBc Ag
Months/2023	Non Reac. (%)	Reactive (%)	Non-Rea (%)	Reactive (%)	Non-Reac. (%)	Non-Reac. (%)	Non-Reactive (%)	Non-Reac. (%)	Non-Reac. (%)	Non-Reac. (%)
January	1.13	0.0	1.13	39.20	17.61	39.20	0,56	0.56	0.0	0.0
February	1.23	0.0	0.23	29.62	11.11	55.55	0.0	0.0	0.61	0.61
March	0.49	1.49	0.0	25.37	19.40	49.25	0.0	0.0	1.49	1.49
Total	1.27	0.42	0.84	31.99	15.88	7.66	0,21	0.21	0.63	0.63

The samples collected from female and male investigated to serum biomarkers of the hepatitis B virus (HBV) showed for all patients with an average proportional of 138.33±54.22 (CV = 0.391) and 137.00±27.83 (CV = (0.203) detected in the 2022 and 2023 years, respectively. The datas are displayed in table 11 and figures 8-9 below.

Table 11 Analysis of means, standard deviation and coefficient of variation of all patients HBV including female and male collected in the 2022 and 2023 years.

Gender	Female	Male	Patients
Year			
2022	83.83±41.66 (0.497)	54.5±13.82 (0.253)	138.33±54.22 (0.391)
2023	75.33±7.76 (0.103)	61.66±22.03 (0.357)	137.00±27.83 (0.203)

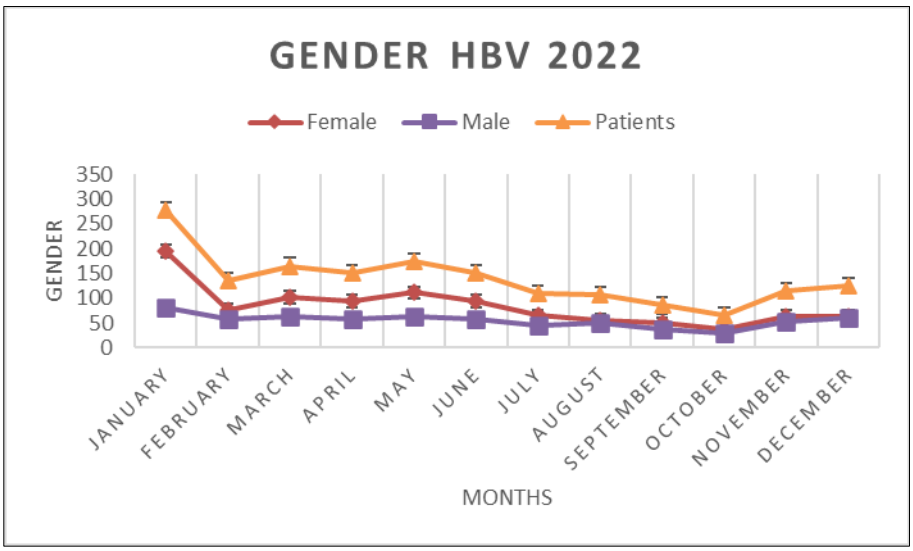

Figure 8 Samples collected among female and male investigated serum biomarkers of the hepatitis B virus (HBV) investigated in the 2022 year.

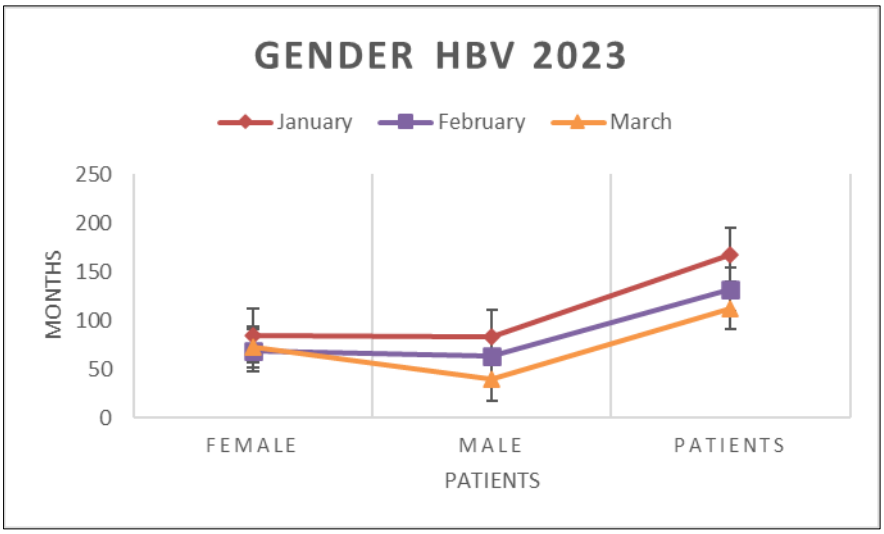

Figure 9 Samples collected among female and male investigated serum biomarkers of the hepatitis B virus (HBV) investigated in the 2023 year.

4. Discussion

The authors suggest follow-up with new serological research associated with molecular assays aimed specifically at reactive and in conclusive results. In addition, the expression of simultaneous positive results indicating the presence of IgM antibodies for different etiological agents does not rule out the possibility of cross-reaction between them. As a result of this work added to the effectiveness of the quality management system, and the internal audits were able to mitigate the critical stages of the organizational process with a focus on risk management. HBV/HCV coinfected patients need more attention. People testing positive for HBsAg or anti-HBc should be monitored while receiving HCV treatment [7]. New research about immunological serum markers should be better investigated to correlation the natural history of hepatitis viral infections with findings (HBeAg seroconversion and HBsAg serum clearance) [2] and improve epidemiological surveillance in clinical trials. In addition, also hepatocellular carcinoma (HCC) antiviral therapy reflects the new challenges in the tumor genomic surveillance and predictive genetic factors associated with risks in the development and worsening of liver injuries and damage of this chronic patients.

5. Conclusion

The authors conclude from the results obtained in this study, the importance of serological markers of viral infections both for screening asymptomatic patients and for monitoring. These individuals are often overlooked and are sources of viral transmission. And the need for in-depth investigation of cases considered positive with viral load from hepatitis infections and whether there is a correlation between the viruses in some specific cases. So, Dra Simões had investigated the improvement of the vaccine model of heterological antigens based on hepatitis B virus envelope protein containing HCV antigen as a chimeric vaccine to the advance of genetic engineering for hepatitis. New research about immunological serum markers should be better investigated to correlation the natural history of hepatitis viral infections and hepatocellular carcinoma (HCC) improve tumour genomic and epidemiological surveillance in clinical trials.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

Statement of ethical approval

Ethical clearance was obtained from the ethical review committee of Department of Sciences Medicine University in accordance with ethical principles for the guidance of physicians in medical research.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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