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Hepatitis B virus (HBV) serum biomarkers for screening and monitoring infections at Rio de Janeiro, Brazil

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Abstract

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. This paper was aimed to investigate the blood-based serum biomarkers for screening and monitoring of Brazilian patients collected in the All Lab at Rio de Janeiro city. This research were carried out using the chemiluminescence method. The presence (reactive) or absence of anti-HBV antibodies (non-reactive) was also investigated by the electrochemiluminescence. The serological diagnosis of hepatitis B involves the evaluation of antigens and antibodies. Our findings had been showed which it is possible to determine the different phases of the infection (reactive – positive or non-reactive – negative) and some samples can be resulted indeterminates or inconclusive by the choice method and the immune system or susceptible of the patients. The sum of numbers, average, standard deviation and coefficient of variation were calculated among the 2222 samples collected from 1660 patients and 477 samples from 411 patients from the year 2022 and 2023, respectively. So, irrespective of HBV genotype or infection phase, this paper provides a classic way fast-track to advance the HBV serum biomarkers detected in blood samples. Novel challenges of serum HBV biomarkers should be more discussed to mitigated weaknesses points and eliminate cross-reaction in clinical assays.

Keywords: HBV; Biomarkers; Liver disease; Serological assays; Screening and monitoring infections.

1. Introduction

1.1. Hepatitis B virus (HBV)

Hepatitis B virus (HBV) is classified in the *Hepadnaviridae* family divided into two genera: *Orthohepadnavirus* and *Avihepadnavirus*. HBV genotypes have been described into nine types based on a divergence of more than 8% (A–J, type I is a subtype of type C), and based on a divergence of more than 4% are classified A–D, F, and I genotypes [1, 2, 3].

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. The Pre–S/S ORF corresponds to the Hepatitis virus surface gene (HBsAg). The Pre–S1, Pre–S2 and S regions have three initiation codons in the same reading phase that, after being translated, originate the proteins: L "Large" (400aa), Middle "M" (281aa) and "Small" (226aa) [1]. These three proteins that comprise the HBsAg are found in the serum of infected individuals in two ways: Small glycosylated (GP27) and Small non–glycosylated (P24), Middle glycosylated (GP33) and Middle di-glycosylated (GP36), and Large glycosylated

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(GP42) and Large non–glycosylated L (P39). HBV B 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 [2, 4].

It is estimated that about 350million people worldwide are carriers of the HBV and approximately 2 million are infected individuals in Brazil. The first reports of variability of the Hepatitis B virus (HBV) were described by Le Bouvier (1971), who identified two mutually exclusive antigenic determinants, d and y, located in HBsAg. Two additional determinants, w and r, were subsequently enunciated by Bancroft et al. (1972). Thus, nine subtypes have been described: ayw1, ayw2, ayw3, ayw4, adw2, adw4, ayr, adrq+ and adrq–, which share a common conformational epitope present in HBsAg. There are eight HBV genotypes (identified as A–H) that exhibit more than 8% divergence between complete genomic sequences. The analysis of genomic variability of HBV isolates is fundamental for molecular and epidemiological studies [1,2].

Worldwide over the last 30years, HBsAg has been used as a commercial vaccine against hepatitis B. It is best way to prevent HBV among aged 18-59 may get the vaccine [1,4]. The Advisory Committee on Immunization Practices (ACIP) recommends hepatitis B vaccination among all adults aged 19–59 years and adults >60 years with risk factors for hepatitis B [4].

There are several methods for serum HBV RNA quantification utilizes molecular assays as RT-qPCR, RT-ddPCR (Droplet digital PCR), 3′ Rapid amplification of cDNA ends (RACE)-based, QuantGene assays, serum HBV RNA, real-time PCR including commercial RNA assays to detected around 10 copies/mL [5, 6]. In addition, an alternative ELISA-based method (HBV NRAg) has been developed by Beijing Wantai Biological to detect the PreS1 antigen and HBcAg [5].

So, it is of high relevance 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 [7]. Genetic markers (single nucleotide polymorphisms) have been associated with the liver damage progression as chronic hepatitis, cirrhosis, fibrosis, hepatocellular carcinoma (HCC), until recurrent HCC during the years, may be some risks of complications involved in the stages of the natural history of disease [7]. Therefore, host miRNA regulation may indicate the development of fibrosis or HCC [5].

1.2. HBV Infection Screening Biomarkers

1.2.1. Hepatitis B surface antigen (HBsAg)

B virus surface protein can be detected in high serum concentrations during acute infection. So this is the first serological marker to appear around four weeks after exposure to the virus, and declining to undetectable levels within 24 weeks. The presence of HBsAg indicates that the person is infected with the B virus [2].

1.2.2. Total antibodies against the "core" of HBV virus (anti-HBc)

It appears at the beginning of symptoms in acute hepatitis B and persists to chronic infection for whole life. The presence of anti-HBc indicates infection by the B virus, current or previous infections. Anti-HBc is used in screening to detect both the IgG antibody and the IgM antibody. Therefore, it is important to define due to high IgG titers (past infection) or IgM (acute phase) [2].

1.3. HBV Infection Monitoring Biomarkers

On the other hand, there are hepatitis B markers used when the disease has already been detected in the body.

1.3.1. Hepatitis B surface antigen (HBsAg)

This marker appears in the blood during acute infection, generally between 6 weeks and 6 months after exposure to the virus and

1.3.2. Total antibodies against the "core" of the Hepatitis B virus (anti-HBc)

These are antibodies directed to the core of the virus, which may be of the IgG and/or IgM type. In addition to the two mentioned serological biomarkers, the main markers for monitoring infection are [2].

1.3.3. IgM antibody against the "core" of the HBV virus (anti-HBc IgM)

This is a marker indicates acute infection, it is found in serum up to 32 weeks after infection. However, this marker may also be present in the chronic phase when the infection worsens.

1.3.4. IgG antibody against the "core" of the HBV virus (anti-HBc IgG)

It is the marker of past infection that characterizes previous contact with the virus, remaining throughout life in individuals who have been infected by the hepatitis B virus [2].

1.3.5. Antibody against hepatitis B surface antigen (anti-HBs total)

The presence of anti-HBs is usually interpreted as recovery and it is the only antibody that confers immunity against HBV. Anti-HBs is also detected in people immunized against the B virus through vaccine. This marker is generally present between the first and tenth week after the disappearance of HBsAg and indicates active immunity (previous contact with the virus or response to the vaccine), or passive immunity (when anti-hepatitis B immunoglobulin is used or transfer of maternal antibodies during pregnancy [2].

1.3.6. Hepatitis B envelope antigen (HBeAg)

This serum biomarker characterizes the viral replication phase and when reactive indicates high infectivity [2].

1.3.7. Against Hepatitis B envelope (Anti-HBe)

It is appears after the disappearance of HBeAg and indicates the end of the viral replication phase [2].

Actually, 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 researches have investigated the improvement of the vaccine model of heterological antigens based on hepatitis B virus envelope protein containing HCV antigen as a quimeric vaccine [1].

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) [4].

2. Material and methods

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

3. Results

The serological diagnosis of hepatitis B involves the evaluation of antigens and antibodies. Through of the combination of these markers, it is possible to determine the different phases of the infection (reactive – positive or non-reactive – negative) and if the patient is immune or susceptible. Some samples can be resulted indeterminates or inconclusive by the choice method. The sum of numbers, average, standard deviation and coefficient of variation were calculated among the 2222 samples collected from 1660 patients and 477 samples from 411 patients from the year 2022 and 2023, respectively. The result can be interpreted that the greater the value of the coefficient of variation, the greater the relative variability of the data in relation to the average of the values (Table 1 and Graphic 1). 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.

Table 1 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

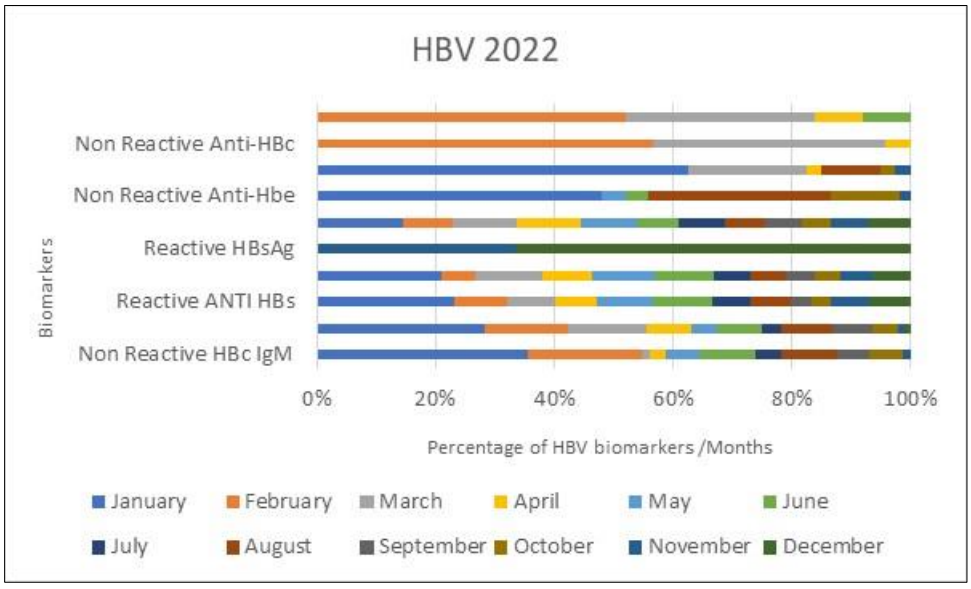

Figure 1 Percentage of HBV biomarkers by months in the 2022 year

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 theses 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 at 2023 (Table 2 and Graphic 2).

Table 2 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 Reac. (%)	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	1.49	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

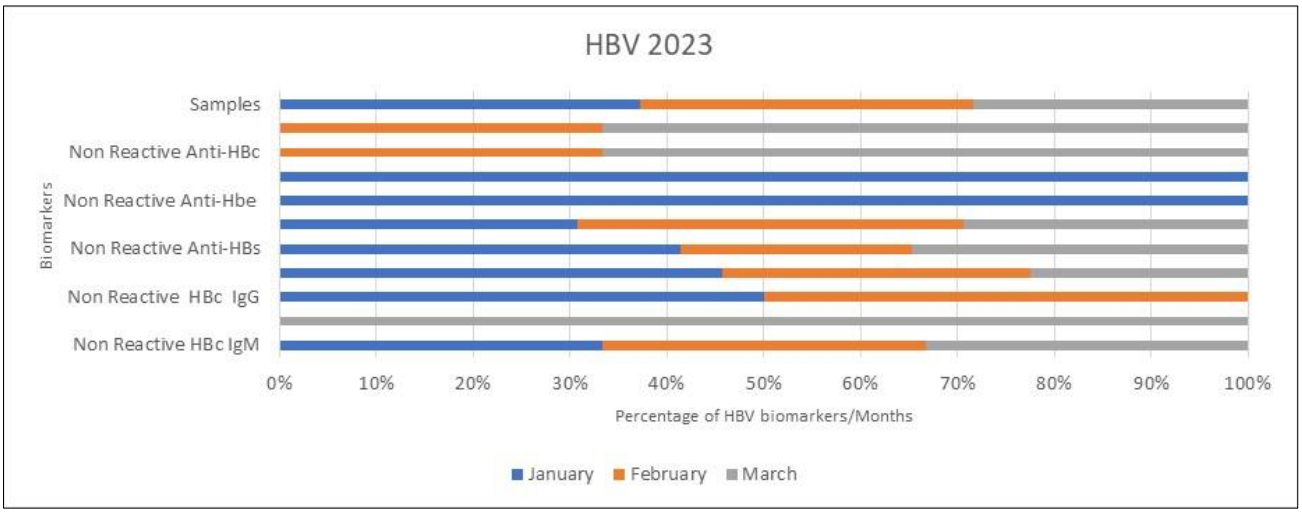

Figure 2 Percentage of HBV biomarkers by months in the 2023 year

It was analyzed the of means, standard deviation and coefficient of variation of HBV bood serum biomarkers collected in the 2022 and 2023 years as showed in table 3.

Table 3 Analysis of means, standard deviation and coefficient of variation of HBV biomarkers in the 2022/2023 years

Biomarkers	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)

On 2022, there was no reactive sample for the biomarker HBc IgM and IgG between the January and December. It had been analyzed about 2222 samples from 1660 patients, which females were more prevalent (60.60%) and males (39.39%) as table 4 and graphic 3-4 below.

Table 4 Percentage of samples 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

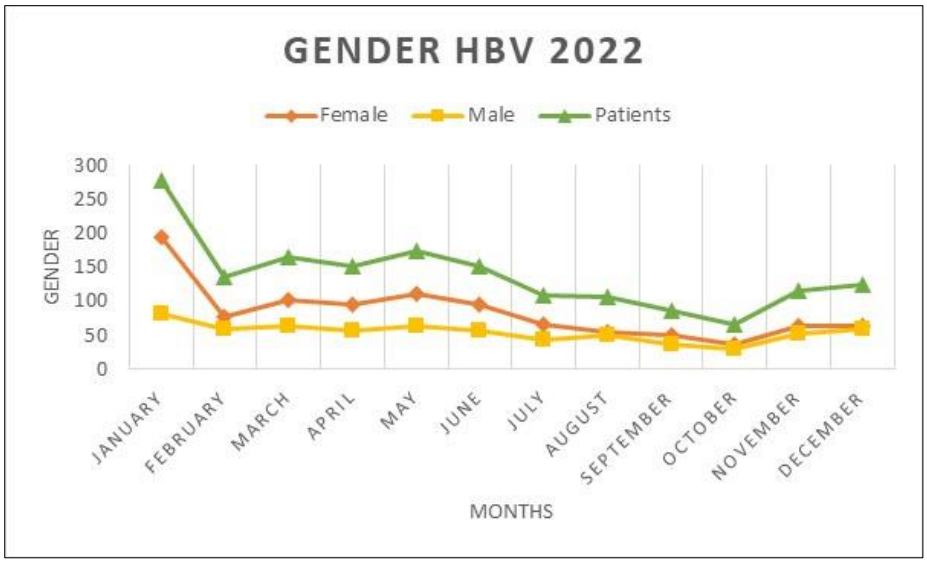

Figure 3 Percentage of samples collected by gender among months in the 2022 years

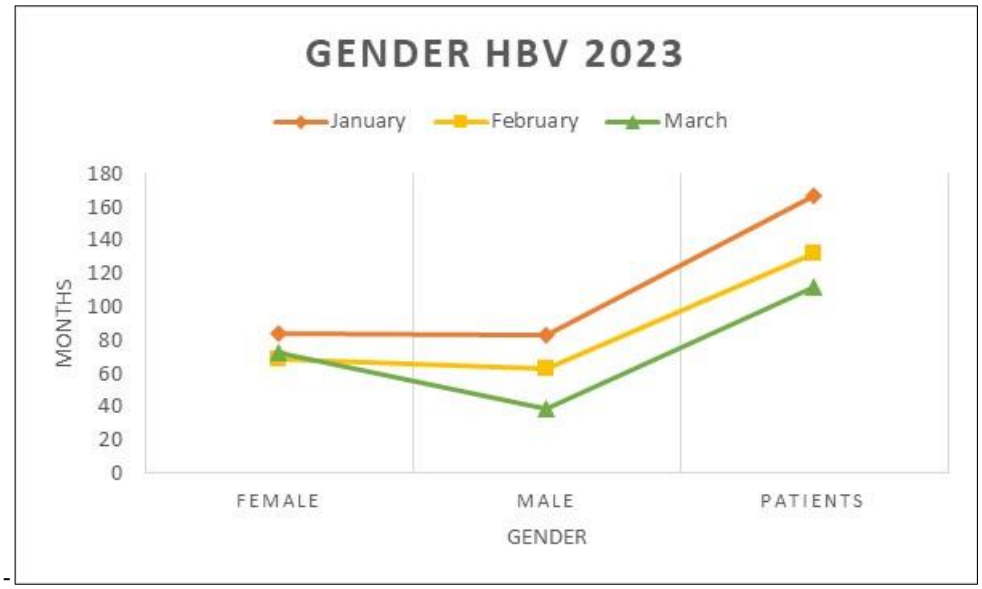

Figure 4 Percentage of samples collected by gender among months in the 2023 years

It was analyzed the of means, standard deviation and coefficient of variation of all patients collected in the 2022 and 2023 years as showed in table 5 below.

Table 5 Analysis of means, standard deviation, and coefficient of variation of all patients including female and male collected in the 2022 and 2023 years.

Year	Female	Male	Patients
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)

4. Discussion

ALL LAB Medical Clinic has developed a high-level structure imposed on all management system standards by International Standards Organization offering alignment with the strategic direction of the organization. Based on knowledge management of resources and change management in organizational planning, All Lab is currently a leader and innovation in health care, being the first clinic at the Rio de Janeiro acting as a reference in support management and recognized for its agility and commitment. In general, 2222 samples were collected from 1660 patients in 2022 and 477 blood samples from 411 patients in 2023, of which the highest prevalence was for the HBsAg biomarker, with the sum of the samples collected, mean, standard deviation and coefficient had been analyzed of variation of all selected biomarkers. In terms of gender, females were more prevalent when compared to samples collected from men in both years investigated. So, irrespective of HBV genotype or infection phase, this paper provides a classic way fast-track to advance the HBV serum biomarkers detected in blood samples at Rio de Janeiro, Brazil. In the context of chronic HBV infection this paper is according the Kramvis and the co-authors (2022) which summarizes an amazing roadmap for emerging serum biomarkers for hepatitis B virus and the importance the new biomarkers to monitor viral as outlook.

5. Conclusion

HBV is a short-term disease and is one of the major causative agents of chronic liver illness. Our findings had been investigated in HBV Infection Screening and Monitoring Biomarkers which it is possible to determine the different phases of the infection (reactive – positive or non-reactive – negative) among the 2222 samples collected from 1660 patients and 477 samples from 411 patients from the year 2022 and 2023, respectively. So, irrespective of HBV genotype or infection phase, this paper provides a classic way fast-track to advance the HBV serum biomarkers detected in blood

samples. Novel challenges of serum HBV biomarkers should be more discussed to mitigated weaknesses points and eliminate cross-reaction in clinical assays.

Compliance with ethical standards

Acknowledgments

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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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