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SAMBUTAN DEWAN REDAKSI

Assalamualaikum Warahmatullahi Wabarakatuh,

Ts di seluruh Indonesia,

Terima kasih atas kesetiaan berlangganan IJCP & ML.

Tajuk (topik) masih berkaitan dengan penyakit jangkitan (infeksi) dan pemeriksaan hematologis, kimia klinis dan imunologis memang merupakan satu kesatuan pemeriksaan bidang Patologi Klinik yang saling berkaitan.

Juga kami ucapkan terima kasih atas naskah calon artikel yang telah dikirimkan untuk penerbitan majalah yang akan datang. Kami mengharap semakin banyak naskah yang dikirimkan guna mengembangkan penelitian ilmu, pengetahuan dan teknologi di lingkup Patologi Klinik.

Wassalamualaikum Warahmatullahi Wabarakatuh.

Dewan Redaksi IJCP & ML

MUTANT HBV INFECTION ON aa143 (T143S)

(Infeksi HBV DI aa143 (T143s))

Maimun Z Arthamin*

ABSTRACT

Lebih dari satu dasawarsa lalu, segi penting mutan virus hepatitis B (HBV) telah teralihkan dari sejumlah kenyataan teori yang tidak diketahui menjadi faktor untuk dipertimbangkan saat mendiagnosis penyakit. Laporan mutan virus hepatitis B (HBsAg) dalam petentu "suatu" telah diperkenalkan. Mutan diisolasi dari penderita seorang laki-laki tanpa gejala berumur 25 tahun, yang ditemukan positif HBsAg tetap, positif untuk antibodi permukaan anti-hepatitis B(anti-HBs) dan negatif untuk kedua penutup lengkap virus hepatitis B antigen (HbeAg) serta penutup lengkap anti-hepatitis B antibodi (anti-HBe). Reaksi rantai polimerase dan urutannya dilakukan serta memperlihatkan genotipe C jenis turunan (subtype) adrq+. Hasil urutan DNA di kawasan "suatu" petentu memperlihatkan adanya mutasi di aa143 (T143S). Yang disajikan ini adalah kasus HBsAg mutan di aa143 (T143S). Sebab "suatu" petentu menunjukkan kawasan imunodominan HBsAg, perubahan sisa dalam "suatu" petentu menjadikan daya antigen merangsang pembentukan zat anti.

Kata kunci: *mutan HBsAg, aa143 (T143S)*

INTRODUCTION

Hepatitis B virus (HBV) infection is very common worldwide, more than one third of the world's population has been infected at some point; and more than 350 million people are chronic carriers.[1,2] HBV infection is associated with different clinical pictures and leads to chronic carrier state in 5 to 10% patients infected in adult life.[1] This immense worldwide reservoir of infection serves as the basis for the generation of HBV mutants because of the unique molecular biology of this virus.[3]

Since the late l980s, we have seen the emergence of mutants across the entire HBV genome as the virus responds to selective pressures, such as vaccination and antiviral therapy. Viral adaptation through mutation will continue as new treatment options are employed and current treatment options are expanded into areas of endemic infection.[3]

The HBsAg is the major component of the the hepatitis virion envelope. It is 226 amino acid residues long, completely embedded in the P gene region. A key region for HBV antigenicity, called the 'a' determinant, is located in the central region (residues 124–147). Amino acid substitutions in the substitutions in the the S gene of HBV, especially in the 'a' determinant region, have been suggested to affect the antigenicity of the virus and the clinical outcome of the infected patient.[4] The most common mutation in the 'a determinant' domain is G145R, but there are several reports for other mutations in this area, as well.[5]

This is a case of an asymptomatic young male with genotype C subtype *adrq+* HBV infection with uncommon T143S mutation. Understanding of HBV mutant impact on disease diagnosis will pose a challenge to global health care for the foreseeable future. Thus, diagnosticians and the healthcare industry need to increase their awareness of HBV mutants and how these mutants may alter current diagnostic and treatment algorithms.

CASE

A 25 years old male with asymptomatic HBV infection. He went to a physician because of urinary tract infection. There was no history of contact with HBV infection person. The physical examination was within normal limit. The laboratory findings on June 11, 2007 were hemoglobin 15.3 g/dl, HCT 45.3%, MCV 91.3 fl, MCH 30.8 pg, RDW 13.6%, WBC 7,040/μl with differential counting eos 3/bas 0/neu stab 3/neu segment 67/lym 21/mon 6, platelet

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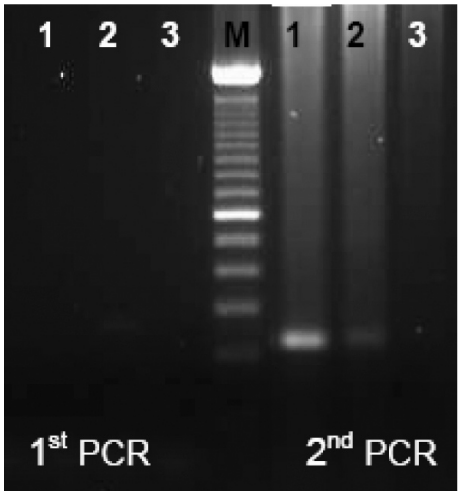
216,000/μl, and ESR 13 mm/h. The urinalysis results were cloudy yellow, SG 1.030, pH 5.0, leucocytes 2+, erythrocytes 2+ eumorphic and others were normal limit. From blood chemistry, the results were the random blood glucose 94 mg/dl, serum uric acid 6.8 mg/dl, AST 25 U/L, ALT 52 U/L, toral bilirubin 0.77, direct bilirubin 0.2, and indirect bilirubin 0.55 mg/dl. The serology of hepatitis B were HBsAg positive S/CO 28.7 (negative if S/CO ≤ 1.000) and anti-HBs positive with titer of 13.1 mIU/ml (negative if ≤ 10.0), both HBeAg and anti-HBe were nonreactive. He was a blood donor in Indonesia Red Cross Blood Bank Malang, East Java, with the laboratory result on July 27, 2005 was HBsAg positive with titer of > 2.50 (cutoff 0.168). In the next blood donation (July 15, 2006), the laboratory result was HBsAg positive with titer of 2.428 (cutoff 0.1443).

On July 3, 2007 HBV DNA detection with nested PCR was negative, but on July 4, 2007 HBV DNA in 2nd nested PCR (gene S 226 bp) was positive, although for the whole gene S (1243 bp) was not detected. The fragment amplified was "a" determinant of S gene.

DISCUSSION

Based on the clinical, laboratory data, and positive HBsAg and HBV DNA, negative HBeAg and anti-HBe, this patient has been suffered from asymptomatic chronic carrier with low level HBV replication. In this case both HBsAg and anti-HBs positive, where anti-HBs usually not detected when HBsAg is also present. But in some cases of chronic hepatitis B



Lane 1 : Patient Sample
Lane 2 : Positive Control
Lane 3 : Negative Control
M : DNA Marker 100 bp

Figure 1. 1st nested PCR HBV DNA was negative, HBV DNA in 2nd nested PCR (gene S 226 bp) was positive.

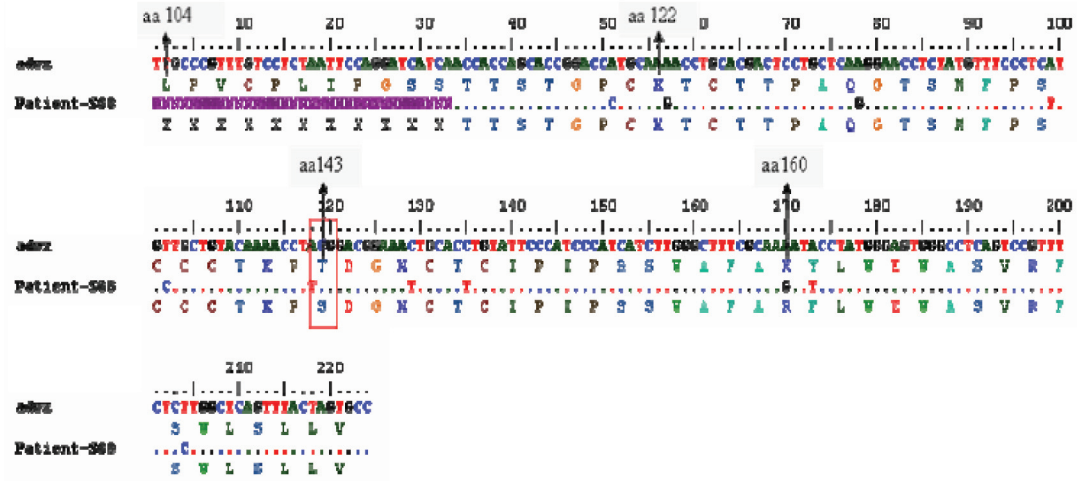


Figure 2. Gene S - HBV DNA analysis. HBV DNA genotyping, Method: Phylogenetic tree based on sequence of S gene region (226 bp). Result Genotype C serotype *adrq+*. HBV DNA analysis PCR and DNA sequencing. Fragment amplified: "a" determinant of HBV DNA (nt 527-596). Result DNA sequencing on a determinant region showed there is mutation on aa143 (T143S)

infection, both HBsAg and anti-HBs can be detected. These antibodies are heterotypic and likely not protective (levels of 10 IU/L) are usually considered protective).[6] In this patient the titer of anti-HBs was > 10 IU/L, another possibility is mixed infection with wild type HBV. But we need cloning to prove it.

HBV was formerly classified into four different subtypes that were afterward subdivided according to the antigenic determinants of HBsAg in adw (adw2 and adw4), ayw (ayw1, ayw2, ayw3, and ayw4), adr (adrq+ adrq–), and ayr.[1,7] Over the last decade, however, subtype determination has gradually been replaced by genotyping.[7] This classification reflecting the phylogenetic origin of the virus isolates was later proposed dividing HBV into eight genotypes, designated A to H, based on divergence in the entire HBV genomic sequence of > 8%.[8]

DNA sequencing of many isolates of HBV has confirmed the existence of multiple viral genotypes, each with a characteristic geographic distribution.[3,9] C strains belong in the indigenous population of Southeast Asia. However, genotype C is also found in the populations of the South Pacific islands. Interestingly, it is possible to differentiate genotype C strains geographically by subtype. The genotype C strains isolated from the Pacific islands are more often of the adrq subtype, as compared to those strains from Southeast Asia.[7] This diversity of the HBV genome is generated by the same mechanism that drives the emergence of mutants, replication.[3] Early studies demonstrating subtype-related clinical differences include the association of Gianotti's disease with subtype ayw in Japan and a higher frequency of liver dysfunction in adr-infected patients compared to those infected with adw. Taking into account that genotype C strains are most often of subtype adr, the latter results have been confirmed by several studies of Southeast Asian chronic carriers.[7]

Hepatitis B virus (HBV) genotypes have been shown to influence the disease profile of chronic hepatitis B infection in some recent studies. In Asia where HBV genotypes B and C are commonly found, the natural history of patients with genotype B differs from those with genotype C.[10] There is a correlation between genotype-subtype and fulminant hepatitis, cirrhosis, HCC, and response to the interferon treatment, where the patients with cirrhosis and HCC older than 50 years genotype C was more prevalent. The genotype C may lead to more severe disease than others and had lower response to interferon.[1,7,8] Different from references, this patient is young adult with asymptomatic chronic carrier, with normal liver function tests.

The HBV genome is a very compact circular genome, with four partially overlapping open reading frames (ORFs). The preS–S (presurface–surface) region of the genome encodes the three viral surface antigens. The most abundant protein is the 24-kD S protein (which is known as HBsAg). The preC–C (precore–core) region encodes hepatitis B core antigen (HBcAg) and hepatitis B e antigen (HBeAg). The P coding region is specific for the viral polymerase. The X open reading frame encodes the viral X protein (HBx).

Hepatitis B viral mutants can emerge in patients as a result of selection pressure from either immune response or treatment options. Mutations that occur within the immunodominant epitopes of hepatitis B surface antigen (HBsAg) allow mutant virus to propagate in the presence of a neutralizing immune response, while wild-type virus is reduced to undetectable levels.[3]

Escape mutants of HBV which have point mutations in the S gene resulting in amino acid changes for the loss of the group-specific determinant called "a" have been reported. They arise in persons infected with HBV after they receive hepatitis B vaccine or in patients with orthotopic liver transplantation on therapeutic trials with monoclonal antibody to HBsAg. HBV escape mutants are reported, also, in a carrier who did not receive hepatitis B immunoglobulin or vaccine.[11]

Mutations in the "a" determinant domain can result in viral escape from the humoral immune response, as the virus may not be recognized by anti-HBs antibodies. Although not frequent, mutants with mutations in the S gene affecting the expression of group-specific determinants of HBsAg are reported.[11] The potent B-cell epitope of HBsAg is borne by aa 124 to 147 of the S gene product, which, it is proposed, make two loop structures maintained by a disulfide bond between Cys-124 and Cys-137 as well as between Cys-139 and Cys-147.[11,12]

Many studies have pointed out that replacement at some amino acid sites could affect the antigenicity of the HBsAg, resulting in the loss of recognition by antibodies and leading to evasion of the virus from the neutralizing antibody response. Since most reported amino acid substitutions have been found within the second of the two loops of the 'a' determinant, the second loop has been considered important; in particular, sites 141 to 145 are thought to be essential for antibody binding. Four amino acid sites–126, 129, 140 and 143–were predicted to be exposed to the surface of HBsAg.[4]

However, amino acid substitutions within this region of the surface protein of the virus, particularly in the region of amino acid 137–147 allow replication of hepatitis B virus in vaccinated subjects, since antibodies induced by current vaccines do not recognize crucial changes in the surface antigen domain.[13] Natural variation and mutations can induce HBsAg conformational changes.[14]

Escape mutants with mutations in the S gene would pose a substantial risk to the community, because current hepatitis B immunoglobulin and vaccines are not effective in preventing infection with them. Blood units containing such mutants are missed by routine screening for HBsAg and can transmit HBV infection to recipients.

HBsAg mutant investigation should be considered when unusual serologic profiles occur, e.g., for (i) individuals with isolated anti-HBc reactivity, (ii) patients with discordant results between HBsAg assays, (iii) patients seronegative for HBsAg but positive for HBeAg, and (iv) individuals with the presence of both HBsAg and anti-HBs (mostly at low titers of < 100 mIU/ml). Moreover, we suggest that since they do not detect the more common G145R and S143L mutants, HBsAg assays using a single monoclonal antibody directed against the second loop of the "a" determinant (amino acids 139 to 147) cannot be used (i) for HBV infection screening of the blood donor population or of organ and tissue donors when anti-HBc testing is not performed or (ii) for systematic screening of HBV infection in pregnant women due to the risk of mutants causing false-negative results.[14]

CONCLUSION

We report and characterize a unusual mutant of the HBsAg. The mutant was isolated from an asymptomatic patient who was found to be persistently positive for both HBsAg, anti-HBs, and HBV DNA and neagtive for both HbeAg and anti-HBe. Due to the unusual immune serological profile, polymerase chain reaction and sequencing were performed and revealed a genotype C. Aligned with known HBsAg sequences from GenBank, this HBV matched to consensus subtype adrq+ and revealed one mutation position within the group-specific "a" determinant region (T143S). These genotype and mutation may impact on clinical course, prognosis, vaccination and treatment options.

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