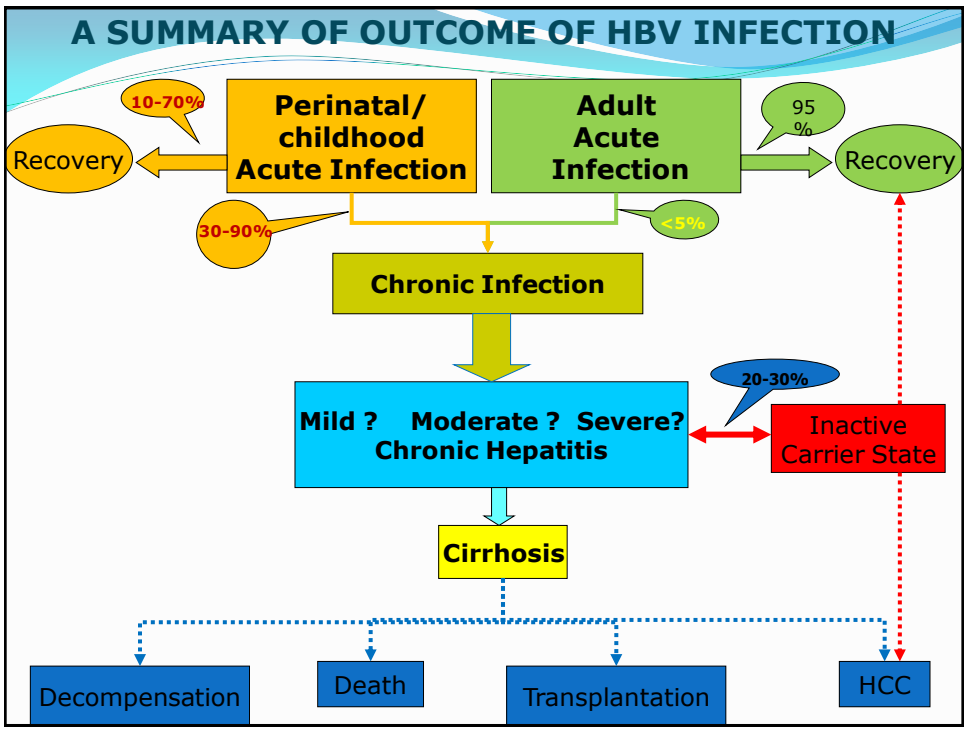
Pathogenesis of Occult Hepatitis B Infection (OBI)

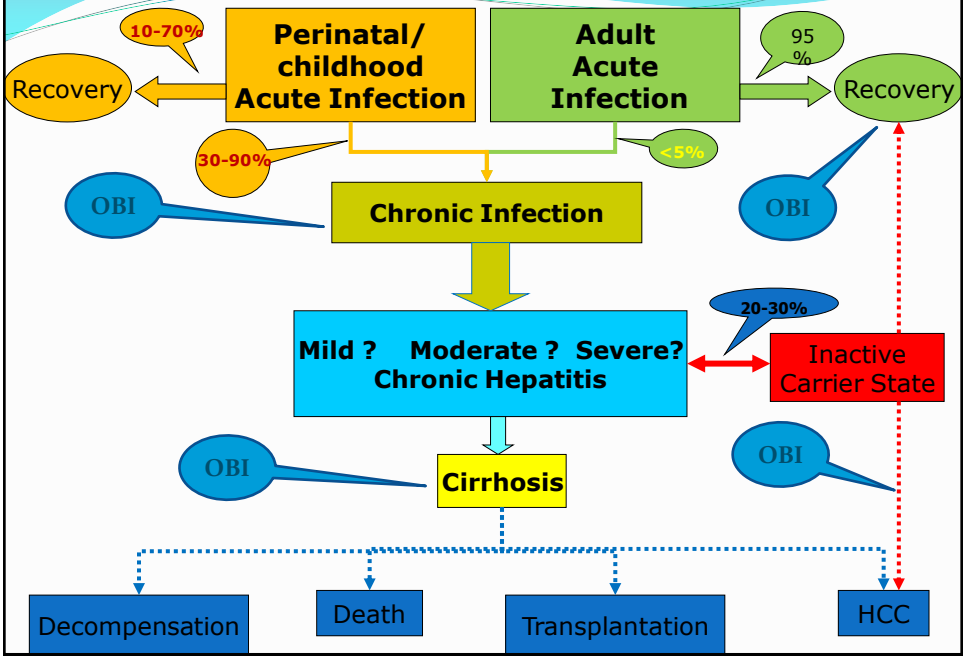
Dr Seyed Mohammad Jazayeri
MD, PhD Clinical Virologist
Hepatitis B Lab-Dept Virology
Tehran University of Medical Sciences



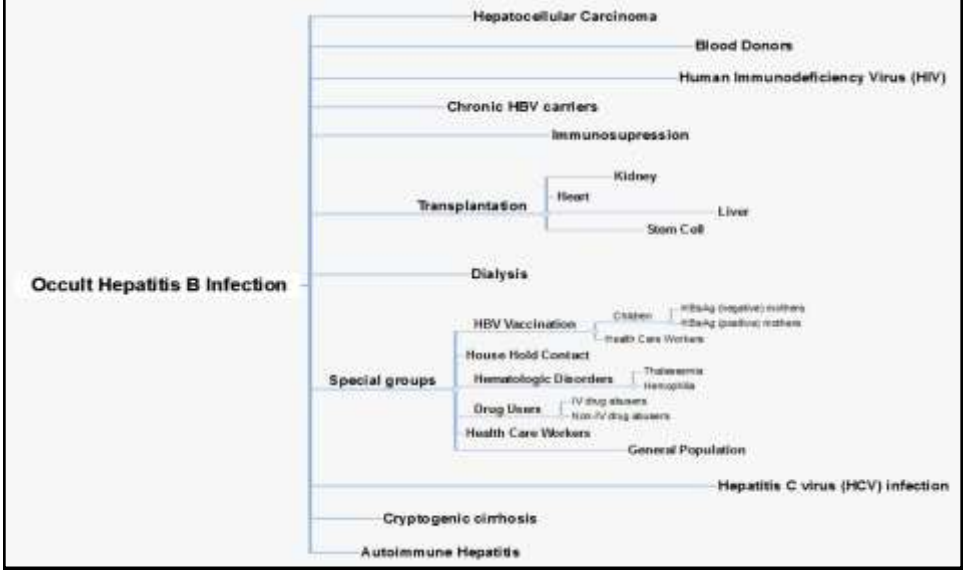
OBI Definition

- Occult Hepatitis B infection is defined as: "Detectable HBV DNA Among Patients Negative For HBsAg".
- Occult HBV infection had not been well studied until HBV polymerase chain reaction (PCR) became available.

A SUMMARY OF OUTCOME OF HBV INFECTION



A schematic phylogenetic tree showing the association of occult hepatitis B in different clinical settings .
Alavian & Jazayeri et al, Hepatitis Monthly, .2012



EPIDEMIOLOGY OF OBI

Reported prevalence of OHB in different clinical settings .Alavian & Jazaveri et al. Hepatitis Monthly. 2012.

Clinical Setting	OHB Prevalence (%)
Blood Donors	0.05%-13%
HIV	0%- 89%
HCV	6.7%-91 1%,
HCC	12%-80%
Immunosuppression	3.3%-37.8%
Dialysis	0%-58%
Chronic HBV carriers	5%-55%
Cryptogenic cirrhosis	4.8%-40%
Transplantation Liver Stem Cell Kidney	36%-64% 0%-50% 0. %-3.3%
HBV vaccinated	2.7%-28%
Family contact of HBsAg positive carriers	8.8%- 28.8%
General Healthy Population	0.7%- 34%
Haemophilia	5.3%- 51.2%

Does anti-HBc positivity could be a surrogate marker for OBI diagnosis?

Clinical Setting	OHB Prevalence (%)	Prevalence of OHB in Anti-HBc positive patients (%)
Blood Donors	0.05%-13%	0%-17%
HIV	0%- 89%	9%-44%
HCV	6.7%-91 1%,	28%-71%
HCC	12%-80%	28.8%-64%
Immunosuppression	3.3%-37.8%	37.8%-62.3%
Dialysis	0%-58%	6.4%-64.7%
Chronic HBV carriers	5%-55%	7%-60%
Cryptogenic cirrhosis	4.8%-40%	17.8-100%
Transplantation Liver Stem Cell Kidney	36%-64% 0%-50% 0. %-3.3%	3%-100% 4.4%-100% 3%-10%
HBV vaccinated	2.7%-28%	6.5%-100%
Family contact of HBsAg positive carriers	8.8%- 28.8%	23.6%- 96.4%
General Healthy Population	0.7%- 34%	6.1%- 51%.
Haemophilia	5.3%- 51.2%	6%-100%

- The percentage of OBI among different clinical settings depends on:

1. Methods of DNA detection
2. Patient recruitment
3. Rate of HBV endemicity
4. Nature of biological material tested

CLINICAL RELEVANCE

Transmission of occult HBV infection

- Carriers of occult infection may be a source of HBV transmission in the case of blood transfusion with the consequent development of a typical type B hepatitis in the recipients.

Reactivation of occult HBV infection

- Immunosuppression induced by:

1. HIV infection
2. Organ transplantation
3. Any patient with occult HBV receiving systemic chemo-radio- or immun otherapy.
4. Hemato-oncologic patients

leading to the development of a typical hepatitis B that often has a severe – and sometimes even fulminant – clinical course.

Occult HBV infection and HCC

- Occult HBV infection is a risk factor for HCC development.
- Three follow up studies showed the role of OBH in HCC development .
- The prevalence of occult HBV among HCC patients varies from 12% to 80% depending on the study.
- OBI increases the rate of HCC up to 3.7%.

OBI DIAGNOSIS

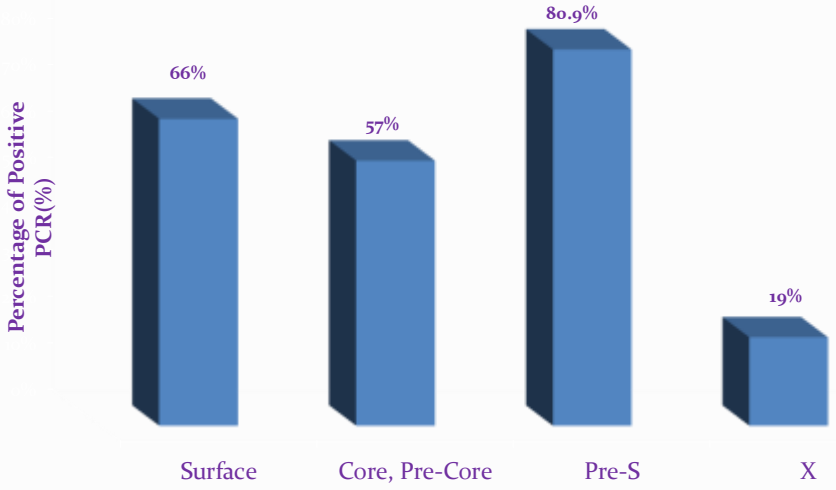
Viral Load In OBI

- During occult infection HBV viral load is usually low with less than 10^4 copies/ml.
- However, the occult HBV viraemia seems to fluctuate over time and remains at a higher level in the liver versus serum in comparison to HBV chronic carriers.

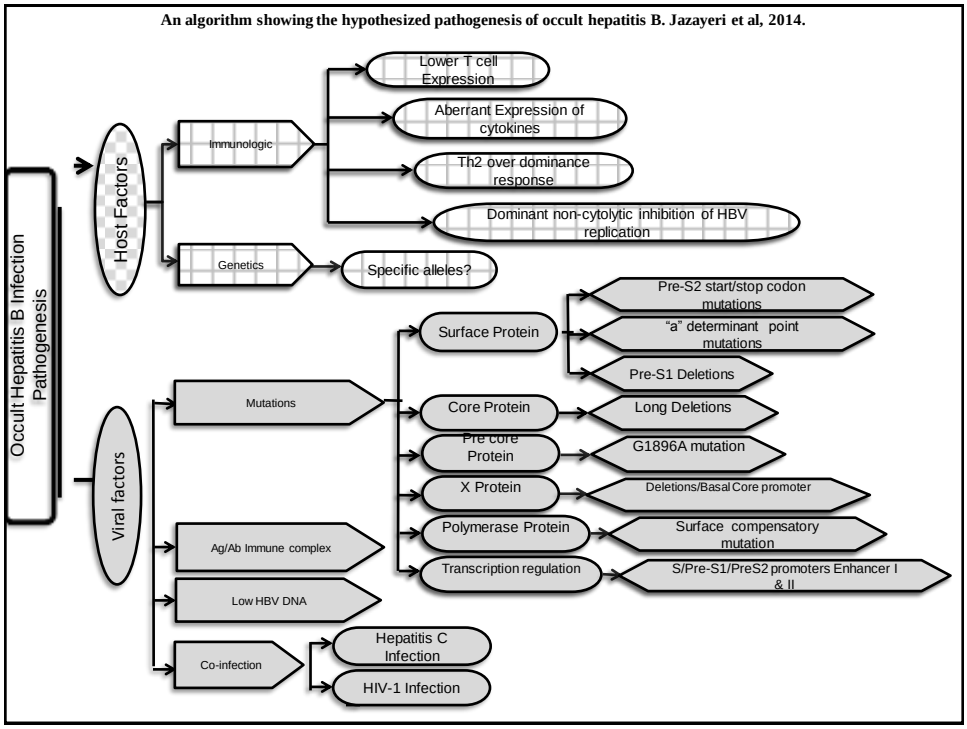
OBI Diagnosis Approach

- The gold standard to test for occult HBV is the analysis of DNA extracts from liver (if not possible) serum samples performed by:
- Real Time technique (as screening tool)
- NestedPCR and the use of oligonucletide primers specific for at least two different HBV genomic regions.

The percentage of positive HBV gene results applying different PCR methods. Shahmoradi et al, 2012



OBI PATHOGENESIS



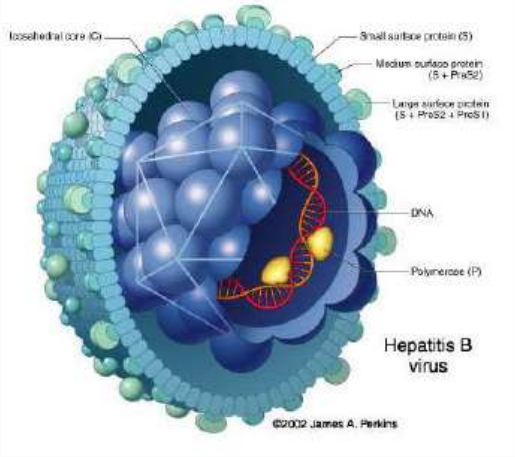
First, HBsAg Mutations

mutations within the surface gene of HBV genome are one of the factors contributing to loss of HBsAg detection by immunoassay (diagnostic/vaccine-escape mutants).

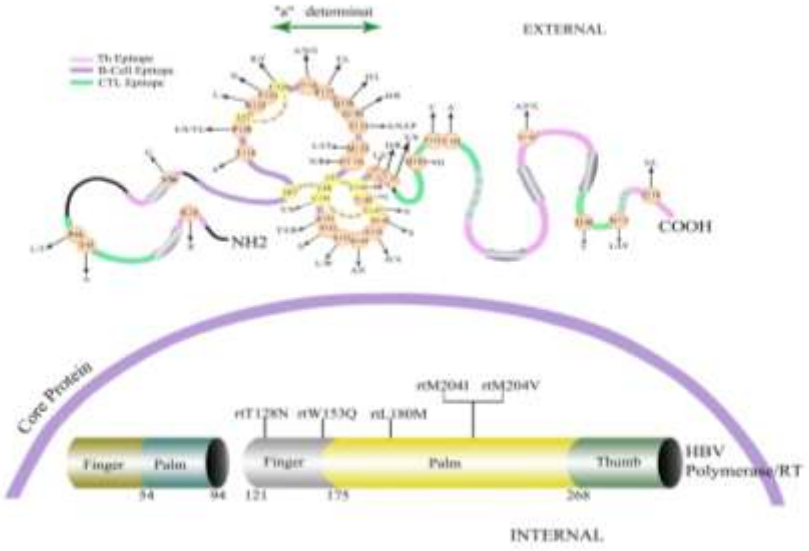
- These "*a*" determinant variants may go undetected by conventional HBsAg screening tests.

20

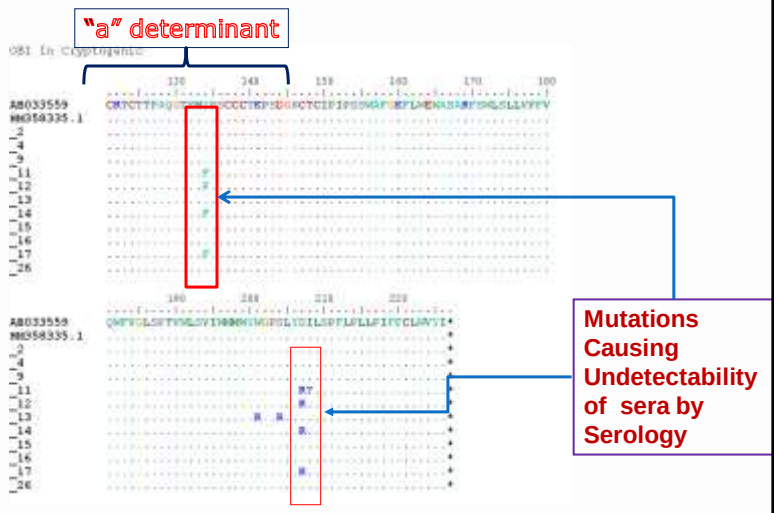
Schematic structure of HBV Dane Particle



Major Hydrophilic Region (MHR) and "a" determinant of Hepatitis B Virus Surface Ag, Jazayeri & Carman, 2012.

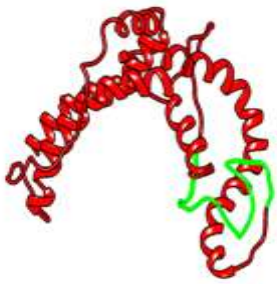


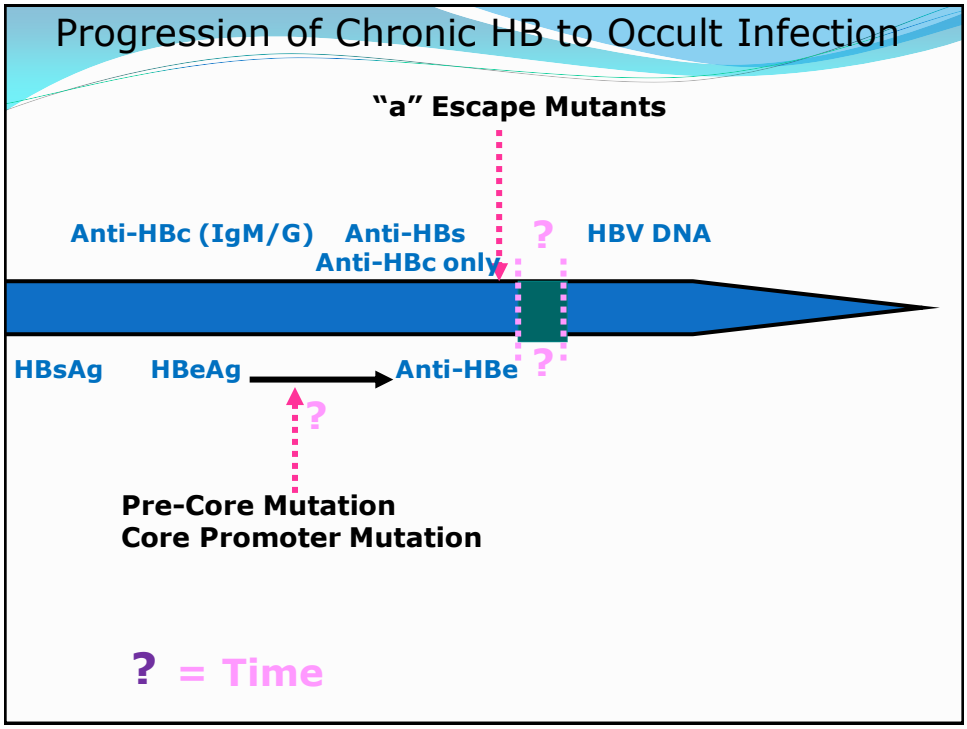
Direct Sequencing Results of HBV surface Protein obtained from Cryptogenic Cirrhosis, Akhavan .2012



Proposed three-dimentional features of HBsAg variants . Sadeghi et al, 2016

Wild type	Mutant (P127L)





Alavian And Jazayeri / Journal of Clinical Virology 57 (2013) 201– 208

Table 3
Variation in sensitivity of HBs antigen detection by various assays. Samples obtained by natural and/or recombinant constructs.

AA position	Reactivity(%)	No. of HBsAg assays	Reference
T118A	19(100%)	19	70,115
P120G/S/L/T	17(68%)	25	75,76,115,126,131
C121S	1(14.2%)	7	126
K/E/R122I	21(67.7%)	31	72,122,126
T123N	23(63.8%)	36	60,72,122,124,126,132
T123A	3	3	133
C124R/Y	6(60%)	10	72,78,122
T125M	3(100%)	3	76
T126S/N	35(100%)	35	115,123–125,131,132
P127T/L	4(80%)	5	76,127
A128T	Weak	3	76
G/Q129H	39(97.5%)	40	115,123–125,132,133
G130D/R/N	6(60%)	10	86,131
T131A/N/I/P	28(84.8%)	33	70,72,78,86,115
M133I/L/T	49(92.4%)	53	60,73,115,123–125,127,131,132
Y134S/L/N	9(100%)	9	72,131
P135S	7(77.7%)	9	115
C137W	3(75%)	4	72
C138R	1(50%)	2	78
C139Y	3 75(%)	4	72
T/L/K140I/Q/E	11(73.3%)	15	72,78,115
P142L/S	19(76%)	25	72,115,127
143L	2 (%)/1 weak	3	131
D144E/A/G	49(80%)/2 weak	61	34,60,70,72,115,122–125,132
G145R	37(54.4%)/10 weak	68	60,78,86,115,123–126,131–135
G145A	9(75%)	12	115,127
G145K	6(75%)	8	72,122
C147S	3(75%)	4	72
T123N/T143S	3	5	124
T126S+G145R	1(20%)/4 weak	5	75
T131N G145R	0	4	86
G130D, G145R	0	4	86
P142L/S-G145R	48(76%)/8 weak	63	72,115,122–125,133
N/DE144A-G145R	9(42.8%)/3 weak	21	115,124,125
T126S/G145R	12(66.6%)	18	115,123,124
Y100C/P120T	1(14.2%)/3 weak	7	60
I195M	2(100%)	2	136
W196 Stop	1(50%)	2	136

Alavian And Jazayeri / Journal of Clinical Virology 57 (2013) 201– 208

Table 3
Variation in sensitivity of HBs antigen detection by various assays. Samples obtained by natural and/or recombinant constructs.

AA position	Reactivity(%)	No. of HBsAg assays	Reference
T118A	19(100%)	19	70,115
P120G/S/L/T	17(68%)	25	75,76,115,126,131
C121S	1(14.2%)	7	126
K/E/R122I	21(67.7%)	31	72,122,126
T123N	23(63.8%)	36	60,72,122,124,126,132
T123A	3	3	133
C124R/Y	6(60%)	10	72,78,122
T125M	3(100%)	3	76
T125S/N	35(100%)	35	115,123–125,131,132
P127T/L	4(80%)	5	76,127
A128T	Weak	3	76
G/Q129H	39(97.5%)	40	115,123–125,132,133
G130D/R/N	6(60%)	10	86,131
T131A/N/I/P	28(84.8%)	33	70,72,78,86,115
M133I/L/T	49(92.4%)	53	60,73,115,123–125,127,131,132
Y134S/L/N	9(100%)	9	72,131
P135S	7(77.7%)	9	115
C137W	3(75%)	4	72
C138R	1(50%)	2	78
C139Y	3 75(%)	4	72
T/L/K140I/Q/E	11(73.3%)	15	72,78,115
P142L/S	19(76%)	25	72,115,127
143L	2 (%)/1 weak	3	131
D144E/A/G	49(80%)/2 weak	61	34,60,70,72,115,122–125,132
G145K	37(54.4%)/10 weak	68	60,78,86,115,123–126,131–135
G145A	9(75%)	12	115,127
G145K	6(75%)	8	72,122
C147S	3(75%)	4	72
T125N/T145S	1	1	124
T126S+G145R	1(20%)/4 weak	5	75
T131N G145R	0	4	86
G130D, G145R	0	4	86
P142L/S-G145R	48(76%)/8 weak	63	72,115,122–125,133
N/DE144A-G145R	9(42.8%)/3 weak	21	115,124,125
T126S/G145R	12(66.6%)	18	115,123,124
Y100C/P120T	1(14.2%)/3 weak	7	60
I195M	2(100%)	2	136
W196 Stop	1(50%)	2	136

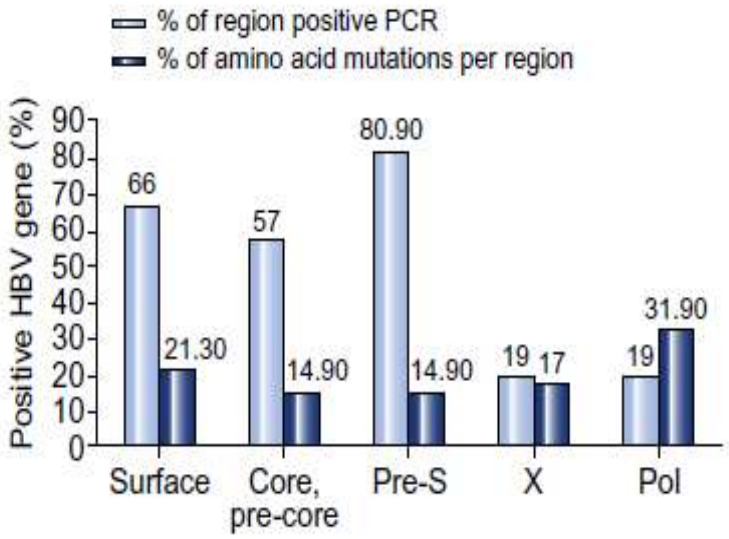
Second, HBV Genome Mutations

However, the failure of HBsAg detection in OBI patients could not be fully explained by surface gene mutations.

- Mutation outside the surface protein may also influence HBV replication capacity. These mutants have also been reported to be less "replication fit" in comparison with wild-type virus.

28

Results of Direct Sequencing of Different HBV Genomes



Third, Low HBV Viral Load

Low level expression of HBsAg due to low viral load might just be enough for viral assembly but is below the sensitivity level of standard tests which making it undetectable.

In our studies, sometimes low level of HBV DNA, rather than genetic variability in the major hydrophilic region (MHR), has been found more frequent among OBI; all isolates had shown DNA level $<10^4$ copy/mL.

30

Demographic, serologic and virologic data of occult HB-positive patients. Shahmoradi et al, 2012.

Sample Code	Age[α]	Sex*	Anti-HBc	Anti-HBs Titer(mIU/mL)	HBV DNA (copy/mL)
14	16	2	+	>100	2100
40	15	1	-	30	2000
42	61	1	-	28	55
46	128	1	-	18	77
52	17	2	-	>100	1270
56	18	1	-	>100	81
65	32	1	-	95	3800
67	38	2	-	38	415
72	37	1	-	>100	223
84	57	1	-	36	9240
86	63	2	-	>100	474
103	12	1	-	>100	468
106	66	2	-	>100	1920
108	35	2	-	>100	347
110	10	1	+	>100	500
112	22	1	-	47	450
115	10	1	+	38	1200
116	64	2	-	25	4560
616	23	1	-	47	2330
122	12	2	+	>100	2300
125	72	2	+	94	395

Fourth , Low HBsAg Synthesis

Due to HBV genetic (mutations in Pre-S region) or viral/host epigenetic factors, the level of HBsAg synthesis decreases to an undetectable levels.

32

Fifth , Ag/Ab Complex

HBsAg and anti-HBs formed complex, and as a consequence the HBsAg was hidden and could not be detected by ordinary serologic assays.

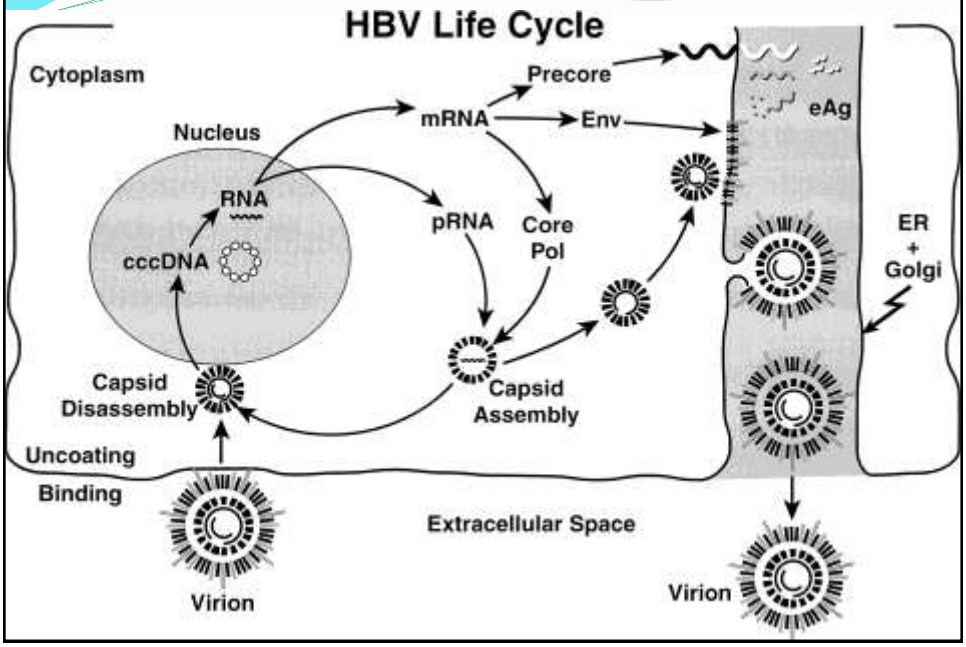
33

Fifth, HBV DNA Persistence

- Follow up studies of patients with HBsAg-positive chronic hepatitis whose serum HBsAg becomes negative, spontaneously or on antiviral therapy, have shown persistance of the viral genome in 28% and 94% in serum and liver, respectively.
- Thus, absence of HBV markers does not exclude the circulation in serum of HBV DNA-containing infectious HBV particles, transmission of which is identical to those in HBsAg-positive sera.

Diagrammatic presentation of HBV life cycle

Taken from Chisari, 1997.



Conclusion

- It is suggested that HBsAg negativity is not sufficient to completely exclude HBV DNA carriers.
- Studies highlights the fact that anti-HBc, anti-HBs and HBsAg may not be effective tools for diagnosis of HBV infection in this high-risk population, and sensitive molecular tests based on real time PCR should be applied for a proper diagnosis.
- The use of sensitive molecular tests such as real-time PCR would be helpful in solving these problems.