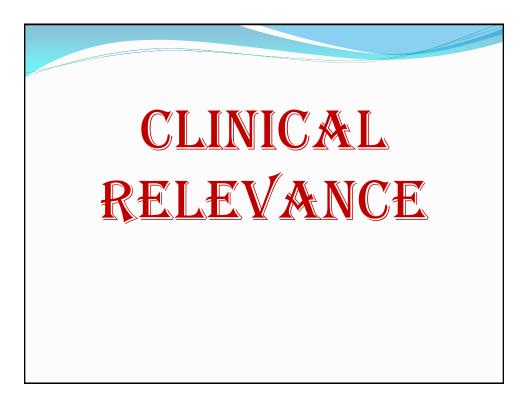


Reported prevalence of OHB in different clinical settings .Alavian &			
Jazaveri et	al, Hepatitis Monthly,	2012.	
Clinical Setting	OHB Prevalence (%)		
Blood Donors	0.05%-13%		
HIV	o%- 89%		
HCV	6.7%-91 1%,		
нсс	12%-80%		
Immunosuppression	3.3%-37.8%		
Dialysis	0%-58%		
Chronic HBV carriers	5%-55%		
Cryptogenic cirrhosis	4.8%-40%		
Transplantation	36%-64%		
Liver	0%-50%		
Stem Cell	0. %-3.3%		
Kidney			
HBV vaccinated	2.7%-28%		
Family contact of HBsAg positive	8.8%- 28.8%		
carriers			
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	o%- 89%	9%-44%		
HCV	6.7%-91 1%,	28%-71%		
нсс	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	36%-64%	3%-100%		
Liver	0%-50%	4.4%-100%		
Stem Cell Kidney	0. %-3.3%	3%-10%		
HBV vaccinated	2.7%-28%	6.5%-100%		
Family contact of HBsAg positive	8.8%- 28.8%	23.6%- 96.4%		
carriers				
General Healthy Population	0.7%- 34%	6.1%- 51%.		
Haemophilia	5.3%- 51.2%	6%-100%		

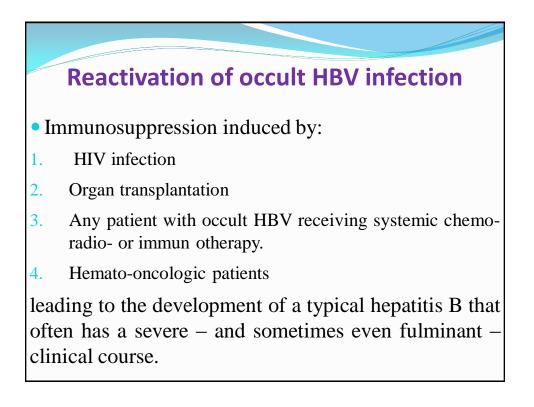
• The percentage of OBI among different clinical settings depends on:

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

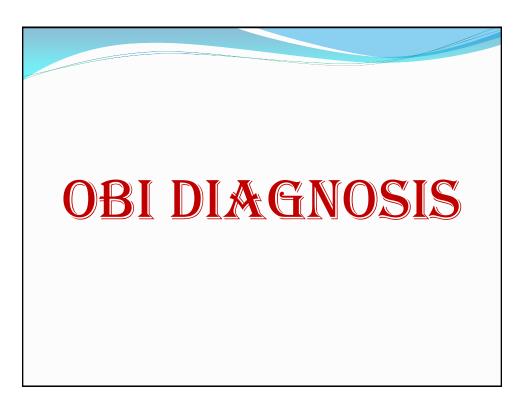


### **Transmission of occult HBV infection**

• 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.



# 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%.

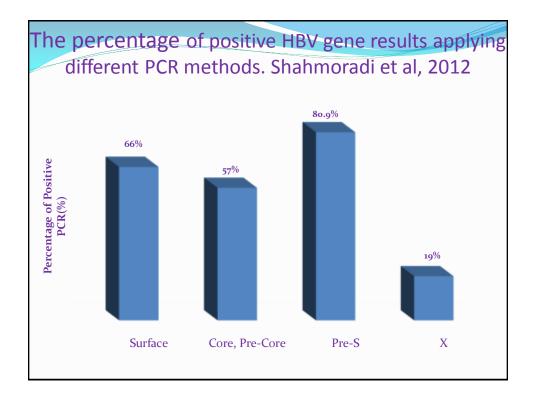


### Viral Load In OBI

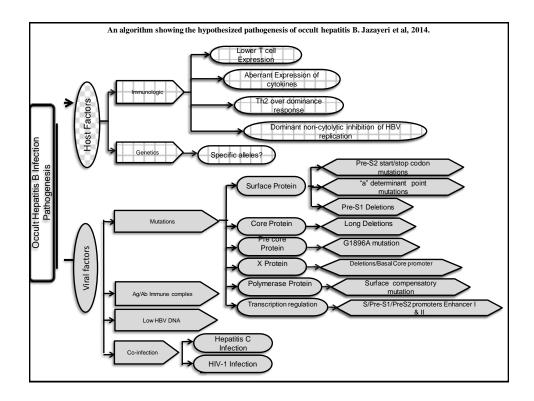
- During occult infection HBV viral load is usually low with less than 10<sup>4</sup> copies/ml.
- However, the occult HBV viraemia seems to fluctuate over time and remains at a higher level in the liver versus serum in comparison to HBV chronic carriers.

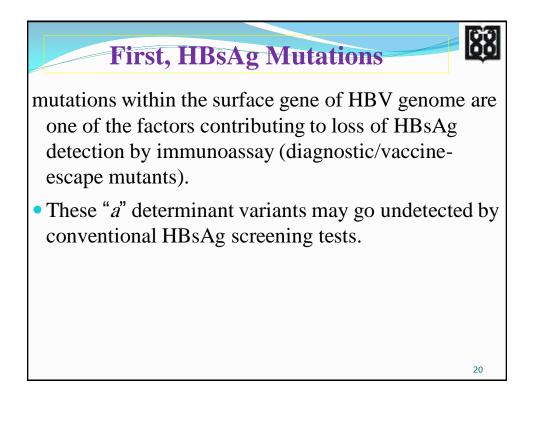
### **OBI Diagnosis Approach**

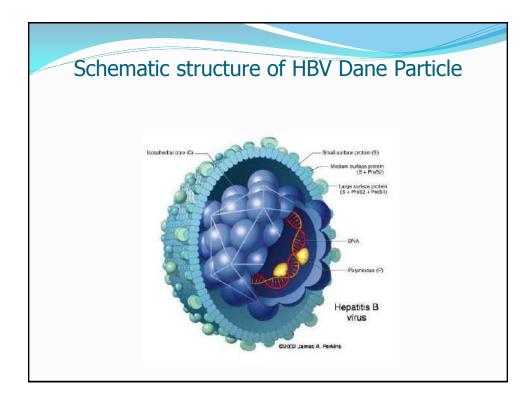
- The gold standard to test for occult HBV is the analysis of DNA extracts from liver (if not possible) serum samples performed by:
- Real Time technique (as screening tool)
- NestedPCR and the use of oligonucletide primers specific for at least two different HBV genomic regions.

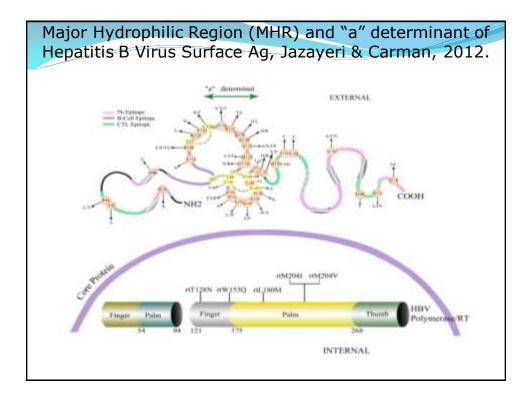


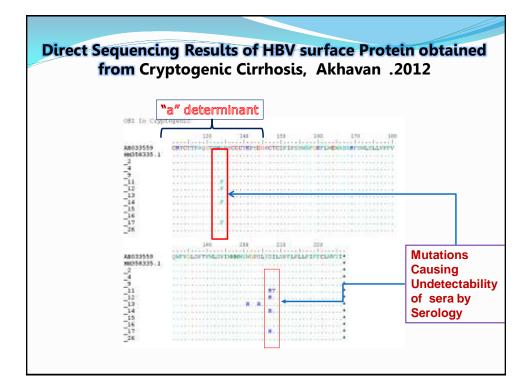


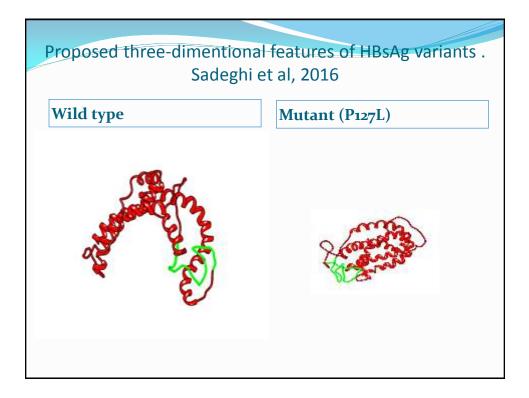


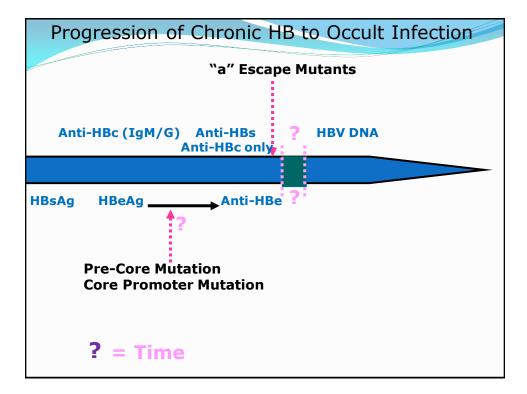












P1206(5)(1 <sup>T</sup> 17988) 25 C1215 1/1425) 7 T123N 24(83.48) 3 T123N 24(83.48) 35 T123A 3 3 T123A 3 C1248(Y 6)(006) 3 T125M 3(1005) 3 T125M 3(1005) 3 T125M 3(1005) 3 T125M 3(1005) 3 T125M 4(005) 4 T125M 4(005) 4 T1	Alavian And Jazayeri / Journal of Clinical Virology 57 (2013) 201– 208 Table 3 Variation is pensitivity of HBs antigen detection by various assays. Samples obtained by natural and/or recombinant constructs.					
P120G_SR_IT         17(988)         25           C1215         1/1420)         3           T122N         21(97.7x)         31           T123N         22(93.8k)         36           T123N         23(93.8k)         36           T123A         3         3           C134RV         6(80.6)         10           T125N         35         3           T125M         3(100.0)         3           T125N         35(100.0)         3           T125NN         35(100.0)         3           T125NN         35(100.0)         3           T125NN         36(00.0)         3           T125AST         Weak         3           T1314/INI/P         28(94.3)         3           T1314/INI/P         28(94.3)         3           T1314/INI/P         28(94.3)         3	sition	Reactivity(CE)	No. of HBsAg assays	Beference		
C125     M(1425)     7       AU(R)22F     21(67,73)     31       M(14)22F     21(67,73)     31       T123A     3     3       C124R(V)     6(100)     10       T125M     3(1000)     3       T125M     3(1000)     3       T125M     3(1000)     3       P127TpL     4(1001)     5       A128T     Weak     3       G1300[KN     6(603)     10       G1300[KN     6(603)     10       G1300[KN     6(603)     10       T135A/N(1/P)     28(84.83)     33       M131L(T)     40(92.43)     33       M131L(T)     40(92.43)     53       Y1345K(N)     9(1005)     9       P1355     7(77.75)     9       C137W     3/7531     4       C138W     15     14       C138W     15     14       P14215     14(75.34)     15       P14215     14(76.54)     25       H31     2(371)     4       C145K     37(54.44)(10 weak     61       G145K     37(54.44)(10 weak     61       G145K     15754)     4       T12M5/G143K     13     12       G145K </td <td>6</td> <td>19(100%)</td> <td>19</td> <td>70,115</td>	6	19(100%)	19	70,115		
NUMBI228         21(97.7%)         31           T123N         23(93.3%)         36           T123N         23(93.3%)         36           T123N         3         3           C124RV         6(00%)         3           T125SN         35(1003)         35           T125SN         35(1003)         35           T125TN         4003()         3           A128T         Weak         3           GQ123H         10(97.5%)         40           GT1200gNn         6(00%)         35           T135A/N(IP)         28(84.3%)         33           M0301LLT         4(92.4%)         53           M0301LT         4(92.4%)         53           T135A/N(IP)         28(84.3%)         3           M0301LT         4(92.4%)         53           V13452,N         9         2           C137W         9         2           C138K         10(90.2% wask         61           D14412/AC         40(90.2% wask         61           C145K         37(5%)         4           C145K         37(5%)         4           C145K         10         3	(S/L/T	17(68%)	25	75,76,115,126,131		
Tižan         20(3.8%)         36           Tižan         3         3           Tižan         3         10           Tižan         3(1003)         3           Tižan         3(1003)         3           Tižan         3(1003)         3           Tižan         3(1003)         3           Tižan         4(1005)         3           Tižan         4(1005)         3           GQ1204H         10(97.5%)         40           GL10D(R)N         6(105)         9           Tižak,Nu/P         2(84.43)         33           Tižak,Nu/P         4(82.43)         33           Tižak,Nu/P         4(82.43)         33           Tižak,Nu/P         8(86.43)         3           Tižak,Nu/P         4(92.43)         33           Tižak,Nu/P         8(86.43)         3           Tižak,Nu/P         8(86.43)         3           Tižak,Nu/P         8(96.25)         9           Tižak,Nu/P         8(96.43)         3           Tižak,Nu/P         8(96.43)         3           Tižak,Nu/P         10(65.5)         9           Tižak,Su/QE         1(77.31)         4		1(14,2%)	7	126		
T122A         3         3           C124R(Y)         Q(URE)         10           T125M         3(1002)         3           T1265(N)         32(1003)         35           T1285(N)         32(1003)         35           T1285(N)         32(1003)         35           T1285(N)         32(1003)         35           A128T         Weak         3           G1300(RN)         6(003)         10           T1314,NIN(P)         26(8433)         33           M1311L(T         46(92,45k)         51           Y13452(L)M         9(1055)         9           P1355         7(77,75)         9           C137W         37(731)         4           C138K         15         9           P142L/5         14(765)         25           I431         2(751)         4           G145K         37(54,110 weak         61           G145K         37(54,110 weak         61           G145K         10(551/2 weak         61           G145K         10(5731)         12           G145K         10(5731)         12           G1445K         10(7533)         4	1226	21(67.7%)	33	72,322,126		
C124Ry         0(000)         10           T125M         3(1000)         3           T125M         3(1000)         35           T125N         35(1000)         35           P1277L         4(000)         5           AT28T         Weak         3           GQ128H         1097-30)         40           GJ100/RN         0(600)         10           T131A/NIP         28(84:83)         33           M131ULT         49(97-48)         53           T131A/NIP         28(84:83)         33           M131ULT         49(97-48)         53           T131A/NIP         28(84:83)         3           M131ULT         49(97-48)         53           T131A/NIP         28(84:83)         3           T131A/NIP         28(84:83)         3           T131A/NIP         28(84:83)         3           C137W         9(72-83)         9           C137W         9(1005)         9           T141K-400(QIE         1(73.38)         4           C138P         15(768)         25           143L         2 (51/1 weak         61           G1442K         3(7583)         8				60,72,122,124,126,132		
T125M         31000         3           T1285JN         35           T1285JN         35           T1285JN         36           T1285JN         35           T1285JN         36           T1285JN         35           T1285JN         36           T1285JN         36           T1285JN         36           T1285JN         36           G1300[RN         6605]           G1301[RN         6605]           T1314,NN/IP         263433)           T134,NN/IP         46952,450           T134,NN/IP         96053)           T134,NN/IP         96053)           T134,NN/IP         97353           T134,NN/IP         97353           T134,NN/IP         97353           G1350         177,73           91355         177,73           9142,JS         177,73           9142,JS         16           1712,NN         17,73           9142,JS         16           1712,NT,4130,DE         17,73           9142,JS         16           164,0         2751           1712,NT,4130,DE         12		3	1	133		
T1265N         35(1005)         35           P1277JL         4(8005)         5           Af28T         Weak         3           GQ228H         31(97.35)         40           G1310D;RN         0(823)         33           M1331LLT         49(92.45)         53           M1331LLT         49(92.45)         53           M1331LLT         49(92.45)         53           M1331LLT         49(92.45)         53           V13455,WA         9         535           V1737,N         9         53           C137W         17531         4           C138R         15981         2           C138P         170,531         4           V11,K140UQIE         1173.332         4           V12,K140UQIE         1173.333         4           V12,K140UQIE         12,53         12           V144L/AC         40(95),2 weak         61           G145K         37,54,8110 weak         61           G145K         37,531         12           G145K         37,531         8           G145K         37,533         4           T120,574,953         12	UY	6(8006)	10	72,78.122		
T12650N         35 (1005)         35           PT22T7L         40005)         5           A128T         Weak         3           CR023PH         30(97.3%)         40           CR023PH         30(97.3%)         40           CR023PH         30(97.3%)         40           CR023PH         30(97.3%)         40           CR023PH         28(84.3%)         53           M123HLRT         48(94.3%)         53           VT345K_VM         9         73(77.7%)         9           VT355         7(77.7%)         9         75(%)         4           C137W         375(%)         4         73(77.7%)         15           P142L/S         11(73.3%)         15         14           P142L/S         12(75%)         25         143L         3           D144L/AC         40(96%)2 weak         61         3           G145K         37(5%)         12         14           G145K         17(7%)         12         14           G145K         37(5%)         4         12           G145K         17(7%)         12         14           G145K         175%)         12 <t< td=""><td></td><td></td><td>3</td><td>26</td></t<>			3	26		
P127T/L         4(00%)         5           A128T         Weak         3           G1301/(KN         0(07.5%)         40           G1301/(KN         0(027.5%)         40           G1301/(KN         0(027.5%)         40           G1301/(KN         0(027.5%)         33           M1331/LT         40(02.4%)         53           Y1345/(KN         9(005)         9           Y1345/(KN         9(005)         9           Y1345/(KN         9(005)         9           C137W         3(77.7%)         9           C137W         3(75%)         4           C138W         15         12           Y13K5/(MQ)E         1(75.3%)         15           P142L5         14(765)         25           G145K         10(75.1%)         15           P142L/G         40(9002)/2 weak         61           G145K         3(75%)         12           G145K         3(75%)         4           C1475         3(75%)         4           T121NG/T445K         10         12           G145K         61         1           G145K         1205(14 weak)         5			35	115.123-125.131.132		
A1287         Weak         3           CQ1230H         30(93758)         40           C130D(R)N         6(80%)         10           T1314/N1(P         28(943%)         33           M123ULLT         40(92,4%)         53           V1345K,NA         9         9           V1345K,NA         9         10           C137W         9         10           C138K         10533         9           C137W         17,73,1         4           C137W         17,73,1         4           C138K         15,933         2           C138W         3,75(%)         4           D1ALKAUQJE         10,73,335         15           P142L/S         12,75(1)         3           D144L[A/G         40(95),2 weak         61           C145K         3,75(%)         12           C145K         10,75(1)         8           C145K         10,75(%)         12           C144K         61         12           C145K         13,75(%)         4           T120K/T145S         3         3           T121K/T145S         3         3           T123K/C145R <td></td> <td></td> <td></td> <td>76,127</td>				76,127		
CQ2 29H         30(97.55)         40           CL30D(R)N         6(005)         10           T31A(N)(IP)         28(84.83)         33           M133L(LT)         4(92.45)         53           Y1345L(M)         9(1005)         9           P1355         7(77.73)         9           C137W         17.7531         4           C1388         1/505)         2           C1389         1/505)         2           C1431         2 (5/1) Weak         3           D144L(A/G         40(902)/2 weak         61           C1455         3(753)         12           C1454         9(753)         12           C1454         9(753)         3           C1454         1/5751         4           C1455         3(753)         12           C1454         1/5751         4           C1455         3(753)         4           C1455         3(753)         4           C1457				76		
C1300/R0N         PC015         10           T131A/N/N/P         28(3433)         33           T131A/N/N/P         28(3433)         33           T131A/N/N/P         28(3433)         33           T134A/N/N/P         28(3433)         33           T134A/N/N/P         8(1005)         9           T134A/N/N/P         9(1005)         9           T134A/N/N/P         9(1005)         9           C137W         1(77,75)         9           C137W         1(77,75)         4           C138R         1(503)         2           C138W         375(15)         4           C139W         375(15)         15           P142L/S         12(501)         25           143L         2 (3)/1 weak         61           C145K         37(54,1)(10 weak         61           C145K         37(53)         12           C145K         37(53,1)         8           C145K         10(5735)         4           T1210FC145R         12(205)/4 weak         5           T131N G145R         0         4           C130D, C145R         0         4           C1405G         4 <td< td=""><td></td><td></td><td></td><td>115,123-125,132,133</td></td<>				115,123-125,132,133		
T131A(N)(IP         28(3438)         33           M1331(L/T         49(92,4%)         51           Y1345(L/N)         9(92,4%)         53           P1355         7(7,73)         9           C137W         1(753)         4           C138R         1(503)         2           C138P         1(753)         4           7(L)K1400(Q)E         1(75,3%)         15           P142L5         10(765)         25           P142L3         10(765)         25           P142L4         2(5)(1) weak         3           D144L2(AG         40(900)(2) weak         61           C145K         3(753)         12           C145K         8(753)         12           C145K         8(753)         12           C145K         8(753)         4           T121N(7143S         3         1           T121N(7143S         3         1           C145K         8(753)         4           C1475         3(754)         4           T121N(7143S         3         1           C1475         1(205)4 weak         5           T131N(6145R         0         4				86,131		
M110/LT         49(92.4%)         53           Y1345/L/N         9(1005)         9           Y1345/L/N         9(1005)         9           P1355         17.7.78)         9           C137W         9(7531)         4           C1388         15(508)         2           C139W         3.75(3)         4           C139W         3.75(3)         4           P142L/S         14(765)         25           P142L/S         14(765)         25           C145K         25(751)         12           G145K         3.75(3,110 weak         61           G145K         3.75(3,110 weak         61           G145K         175731         12           G145K         10(753)         12           G145K         10(753)         4           T121NG/145K         10(753)         4           T1230S-G145R         10(251)4 weak         5           T131N G145R         0         4           G142L/5 GL45R         42(754)8 weak         63           M12L/5 GL45R         9(42)8(3) weak         21           T1280S/G1458         7(4306,33)         16           T1280S/G1458         7				70,72,78,86,115		
Y1345LW         91005)         9           P1355         X(77.76)         9           C137W         X(77.76)         9           C138R         15075)         2           C138P         15075)         2           C138P         17/3.76)         3           P142L95         19(753)         3           D144E(AC         25/543)         3           D144E(AC         400052/2 weak         61           C145R         31/551)         4           C145R         9/753)         12           C145R         9/753)         12           C145R         9/753)         4           T123NC/1452         1         3           C145R         9/753)         8           C1475         3/751)         4           T123NC/1452         1         3           C145R         0         4           C130D, C145R         0         4           V142L/5-C145R         42/753/8 weak         63           NDE144A-G143R         9/42/8/3 weak         63           NDE144A-G143R         9/42/8/3 weak         63           NDE144A-G143R         9/42/8/3 weak         63 <td></td> <td></td> <td></td> <td>60,73,115,123-125,127,131,132</td>				60,73,115,123-125,127,131,132		
PI355         2(77.76)         9           C137W         3(77.76)         4           C137W         3(77.76)         4           C138R         U(508)         2           C138W         375(8)         4           T/1AK1400(pE         11(75.318)         15           P142L/S         14(765)         25           148L         2 (8)(1) weak         3           D14442/MG         40(9005)2 weak         63           G145K         3(7513)         12           G145K         8(7513)         12           G145K         8(7513)         8           C1475         3(7513)         4           T121NG/1445K         1         3           T121NG/1445K         1         3           T121NG/1445K         1         3           T121NG/1445K         1         3           T121NG/1445K         0         4           G130D, G145R         0         4           G1405, Heweak         5         3           T131NG (462R         0         4           M12L/5 (445R)         42(7563)8 weak         63           M12L/5 (445R)         5/42(8)3 weak         63				72.131		
C137W         Y(733)         4           C138R         1(503)         2           C138P         375(%)         4           Y(X)K140(Q)E         1(7)3.3%)         15           P42L/S         11(73.3%)         15           P42L/S         12(76%)         25           143L         2 (%)F weak         3           D144E(A)/G         40(95%)2 weak         61           G145K         37(54.4%)(10 weak         61           G145K         37(54.4%)(10 weak         63           G145K         37(54.4%)(10 weak         63           G145K         37(53.3%)         4           T121NG71435         3         5           T121NG71435         3         5           G130R, G145R         0         4           G130R, G145R         0         4           G130R, G145R         0         4           G130R, G145R         0         4           G130R, G145R         9(42.8%)/3 weak         63           MDE1446-G143R         9(42.8%)/3 weak         21           T12085G1458         7(46.6%)         16				115		
C138R         1598)         2           C139Y         375(x)         4           C139Y         375(x)         15           P142LJS         19(783)         25           148L         215(Y) Iweak         3           D144L[A/G         400003/2 weak         61           C145K         375(1)         12           C145K         8(758)         12           C145K         8(758)         12           C145K         8(758)         4           C145K         12         4           C145K         1375(1)         4           C145K         1375(1)         4           C145K         1375(1)         4           C145K         1225(2)/4 weak         5           C130D, C145K         0         4           C130D, C145K         0         4           V142L/5-C145R         42(767)/6 weak         63           NDE1440-C143R         9/42/83) weak         63           NDE1440-C143R         9/42/83) weak         21           T308/G1498         70(66/87)         16				72		
C1389         3 75(%)         4           7D,DK1400,QF         11(73.3%)         15           9421,JS         11(73.3%)         25           1438         215(7) (waik         3           D14412,IAC         40(963)/2 wask         61           D14412,IAC         31(95.4%) (10 weak)         63           G145K         37(94.4%) (10 weak)         68           G145K         37(95.1%)         12           G145K         16(75%)         8           G145K         17(751)         12           G145K         16(75%)         4           T12205/G145R         12         5           G130D,G145M         0         4           G130D,G145M         0         4           G130D,G145M         9/42,D5/G145K         63           NDE1444-G143K         9/42,05/31 weak         63           NDE1444-G143K         9/42,05/31 weak         21           T1205/G145M         12/06,073         16				78		
TJL JAT SAUQUE         TJ TJ JAT SAUQUE         TJ TJ JAT SAUQUE         TJ TJ JAT SAUQUE         TJ JAT SAUQUE <t< td=""><td></td><td></td><td></td><td>72</td></t<>				72		
Pi 421,15         147,05         25           143L         2 (3,1) weak         3           D1442,A/C         40,050,12 weak         61           C145K         37,054,45,110 weak         61           C145K         37,054,45,110 weak         61           C145K         37,054,45,110 weak         61           C145K         97,753,1         12           C145K         16,753,1         8           C1475         37,753,1         4           T12305-C1458         12,005,14 weak         5           T131N G145R         0         4           G130D, C145R         0         4           M22L5-C145SR         42,7562,16 weak         63           M32L5-G145R         9/42,853,19 weak         21           T3205/C1458         70,66,035         18			15	72.78.115		
HBL         2 (3)/1 weak         3           D144E/A/G         40(920)/2 weak         61           G145E         37(944)/2 weak         63           G145K         37(953)/2 weak         63           G145K         97(753)         12           G145K         97(753)         8           C147S         3/7531         4           T123N(77455         1         5           T131N G145R         0         4           G130D, G145R         0         4           V142L/5-G145R         48(762)/8 weak         5           MDE1444-G143R         9(42.85)/3 weak         63           NDE1444-G143R         9(42.85)/3 weak         21           T2365G1458         16         5				72.115.127		
D144L/A/G         4090203/2 weak         61           G145R         37(54.4K)[10 weak]         68           G145R         37(54.4K)[10 weak]         68           G145K         9(758)         12           G145K         9(758)         8           G145K         9(758)         4           T121NG/T4458         1         5           T121NG/1458         5         1           G145K         0         4           G130D, G145R         0         4           W142L/5 G145R         48(768)[8 weak]         63           NUBE1444-G143R         9(42.85)[3 weak]         21           T12045/G145R         16(66.85)         16				131		
G145K         37(54.4K)/10 weak         68           G145K         9(75%)         12           G145K         9(75%)         8           G145K         9(75%)         4           G145K         9(75%)         4           G1475         3(75%)         4           G1300, G145K         0         4           G1300, G145K         0         4           H142U5-G145K         0         4           H142U5-G145K         9(42,850) weak         63           NJDE144A-G145K         9(42,850) weak         21           T308G76456K         12(66,63)         18				34,60,70,72,115,122-125,132		
CI-45M         9(758)         12           GI-45K         8(758)         8           CI-475         3(758)         4           T12305/CI-458         1         3           T121NC/1425         1         3           C1455         0         4           C1475         0         4           C1475         0         4           C1475         0         4           C1205/C1458         0         4           C13005/C1458         0         4           V142L/5-C1458         8(768)8 weak         63           NUDE1440-C1438         9(42.85)3 weak         21           T12045/C1458         7(06.08)         16				60.78.86.115.123-126.131-135		
G14SK         B(75%)         B           C1475         3(75%)         4           T123Nc71435         3         5           T1245-C1458         1/20%14 weak         5           C1300, C1458         0         4           G1300, C1458         0         4           P142U/5-C1458         42/75%18 weak         5           N/DE144A-G145R         9/42/80/3 weak         63           N/DE144A-G145R         9/42/80/3 weak         21           T236S/C1458         1/2066/81         18				115.127		
C1475         3(75%)         4           T123NFC7455         1         5           T131NC7455         1         5           C130DS-C1458         1/2015/14 weak         5           C130DS-C1458         0         4           C130DS-C1458         0         4           V142L/5-C1458         48(76%)8 weak         63           N(DE1444+C1438         9/42.8%)3 weak         21           T236S/C1458         12(66:08)         18				72.122		
T123DFG12435         3         5           T123DFG12458         t) 2015/4 weak         5           T131N G1458         t) 2015/4 weak         5           G130D, G1458         0         4           W12L/5-G1458         48/7453/8 weak         63           NDE1444-G1458         5/42/85/3 weak         21           T12045/G1458         7/66/683         16				72		
T1285-G1458         1/2013/14-weak         5           T1310-G1458         0         4           G1300-G1458         0         4           I/1421/G-G1458         0         4           I/1421/G-G1458         0         4           I/1421/G-G1458         8/42765/j8 weak         63           N/DE144A-G1458         9/42/80/3 weak         21           T2365/G1458         12/66/08)         18		alizel		124		
T131N G145R         0         4           G13DD, G145R         0         4           M42L/S-G145R         48(763)8 weak         63           NDE1444-G145R         9(42.85)3 weak         21           T305G1458         12(66,03)         16		1		75		
G130D, G145R 0 4 11/42L/5-G145R 48(7453/8 weak 63 N/DE144A-G145R 9/42.85(3) weak 21 17345/G145R 12(06/33) 18		of the Per works		75		
IP142L/S-CI452R         48[7452]8 weak         63           NJDE144A-G1458R         9(42,285)7 weak         21           T2055G1458R         12(66,63)         18		0				
NJDE144A-G1458 9(42,85)(3 weak 21 T1255(G1458 12(66.03) 18		U ANTINA ANTINA ANTINA		86		
T1265(C1458 12(66.03) 18				72,115,122-125,133		
				115.124,125		
				115,123,124		
		1(14.2%)/3 weak	7	60		
1195M 2(1000) 2 W196 Stop 1(50%) 2				136		

able 3 ariation in sensitivity of HBs an	tigen detection by various assays. Samples ob	tained by natural and/or recombinant constru	cts.
AA position	Bractivity/(%)	No. of HBsAg assays	Beference
T110A P120C(5)LT C1215 NITRI228 T123N T123N T123N T125N T125N T125N T125N T125N T125N T125N T125N T125N T125N T125N T125N T125N T125N T125N T135AJNA[T T135A]N T135A]NA[T T135A]N	19(1003) 17683) 1(1425) 21(67.78) 23(63.88) 3 6(605) 3544005 4(003) Weak 39(95.753) 6(605) 28(94.33) 46(92.48) 9(1005) 7(77.73) 9(205) 7(77.73) 1(73.38) 1(505) 7(753) 1(753) 1(753) 1(753) 1(753) 1(753) 1(753) 1(10	19 25 31 36 3 3 10 3 3 5 5 3 40 10 33 35 5 3 40 10 33 5 5 3 9 9 4 2 4 2 3 6 10 33 3 5 5 3 6 10 10 33 5 5 3 6 10 10 33 5 5 5 3 40 10 33 5 5 5 5 3 40 10 3 5 5 5 5 3 40 3 5 5 5 5 5 3 40 3 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	70,113 75,76,115,126,131 126 77,122,126 60,72,122,124,126,132 133 76,115,123,125,131,132 76,115,123,125,131,132 76,131 70,72,78,68,115 60,731,15,123,-125,132,133 86,131 70,72,78,68,115 72,78 72 72 72 72 72 73 73 72 72 73 74 75 72 72 75 72 72 75 72 72 78 72 72 78 72 72 78 72 72 78 72 72 78 72 72 78 72 72 78 72 72 78 72 72 78 72 72 78 72 72 78 72 72 78 72 72 78 72 72 72 72 72,115,127 131 34,64(70,72,115,122)-125,132,135 131 34,64(70,72,115,122)-125,132
T1265+G1458 T13EN G1458 G1300, G1458 P142LJ5-G1458	1/20%1/4 weak 0 0 48(76%3/8 weak	5 4 63	75 86 86 72,115,122-125,133
N/DE144A-G1458 T1265/G1458 Y100C/P120T I195M W196 Stop	9(42,85)(3 weak 12(66,03) 1(14,23)(3 weak 2(1003) 1(505)	21 18 7 2 2	115.124,125 115.121,124 60 136 136

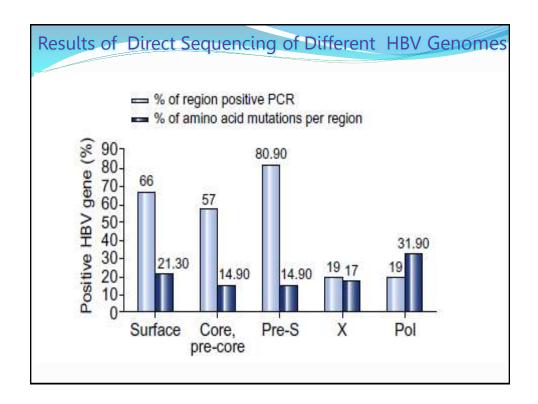
### Second, HBV Genome Mutations

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.

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## 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<sup>4</sup> copy/mL.

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Demogr	aphic, se	erologic	and virolog	ic data of occult HB-p	ositive patients.
			Shahmorad	di et al, 2012.	
Sample Code	Ageα	Sex*	Anti-HBc	Anti-HBs Titer <u>(mIU/mL)</u>	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.

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# 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.

### 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.

