Results

QUANTITATIVE AND QUALITATIVE HBV-DNA BY REAL TIME PCR TOGETHER WITH HEPATITIS B MARKERS

PSer NO	PCR Quantitative	PCR Qualitative	HBcIgG	HBeAb	HBeAg	HBsAg	ALT	AST
1	27 X 10^3 Cop/ ml	Positive	Positive	Positive	Negative	Positive	23	19
2	38 X 10^3 Cop/ ml	Positive	Positive	Negative	Positive	Positive	20	42
3	10 X 10^3 Cop/ ml	Positive	Positive	Negative	Positive	Positive	142	405
4	5 X 10^3 Cop/ ml	Positive	Positive	Positive	Negative	Positive	30	40
5	2 X 10^6 Cop/ ml	Positive	Positive	Negative	Positive	Positive	90	67
6	219 Cop/ ml	Positive	Negative	Negative	Negative	Positive	17	22
7	422 Cop/ ml	Positive	Positive	Positive	Negative	Positive	26	28
8	6 X 10^6 Cop/ ml	Positive	Positive	Negative	Positive	Positive	290	129
9	1960 Cop/ ml	Positive	Positive	Positive	Negative	Positive	7	19
10	2140 Cop/ ml	Positive	Positive	Negative	Negative	Positive	10	18
11	619 Cop/ ml	Positive	Positive	Negative	Negative	Positive	26	21
12	31 X 10^3 Cop/ ml	Positive	Positive	Positive	Negative	Positive	25	19
13	115 X 10^3 Cop/ ml	Positive	Positive	Negative	Positive	Positive	34	36
14	816 X 10^3 Cop/ ml	Positive	Positive	Positive	Negative	Positive	23	14
15	181 Cop/ ml	Positive	Positive	Positive	Negative	Positive	41	27
16	17 X 10^6 Cop/ ml	Positive	Positive	Negative	Negative	Positive	250	18
17	25 X 10^6 Cop/ ml	Positive	Positive	Negative	Positive	Positive	160	120
18	250 X 10^3 Cop/ ml	Positive	Positive	Negative	Positive	Positive	100	80
19	1800 Cop/ ml	Positive	Positive	Positive	Negative	Positive	25	21
20	690 X 10^3 Cop/ ml	Positive	Negative	Positive	Negative	Positive	85	65

Table (2)

PSer NO	PCR Quantitative	PCR Qualitative	HBcIgG	HBeAb	HBeAg	HBsAg	ALT	AST
21	12 X 10^6 Cop/ ml	Positive	Positive	Negative	Negative	Positive	41	29
22	181 X 10^3 Cop/ ml	Positive	Positive	Negative	Positive	Positive	290	129
23	478 X 10^3 Cop/ ml	Positive	Positive	Negative	Positive	Positive	200	180
24	5 X 10^3 Cop/ ml	Positive	Positive	Negative	Negative	Positive	19	7
25	1.8 X 10^3 Cop/ ml	Positive	Positive	Negative	Negative	Positive	90	67
26	4.3 X 10^6 Cop/ ml	Positive	Positive	Negative	Positive	Positive	142	405
27	17 X 10^3 Cop/ ml	Positive	Positive	Negative	Positive	Positive	42	20
28	816 X 10^3 Cop/ ml	Positive	Positive	Positive	Negative	Positive	14	23
29	800 X 10^3 Cop/ ml	Positive	Positive	Negative	Positive	Positive	120	187
30	15 X 10^3 Cop/ ml	Positive	Positive	Positive	Negative	Positive	34	36
31	18 X 10^3 Cop/ ml	Positive	Positive	Negative	Positive	Positive	30	37
32	205 Cop/ ml	Positive	Negative	Positive	Negative	Positive	22	19
33	800 Cop/ ml	Positive	Positive	Positive	Negative	Positive	30	18
34	20 X 10^6 Cop/ ml	Positive	Positive	Negative	Positive	Positive	67	151
35	460 Cop/ ml	Positive	Positive	Negative	Negative	Positive	17	25
36	38 X 10^3 Cop/ ml	Positive	Positive	Positive	Negative	Positive	60	57
37	32 X 10^3 Cop/ ml	Positive	Positive	Negative	Positive	Positive	40	19
38	3.7 X 10^6 Cop/ ml	Positive	Negative	Negative	Positive	Positive	44	57
39	20 X 10^6 Cop/ ml	Positive	Positive	Negative	Positive	Positive	48	66
40	16 X 10^6 Cop/ ml	Positive	Positive	Negative	Positive	Positive	240	187

PSer NO	PCR Quantitative	PCR Qualitative	HBcIgG	HBeAb	HBeAg	HBsAg	ALT	AST
41	2.4 X 10^3 Cop/ ml	Positive	Positive	Negative	Negative	Positive	29	32
42	120 X 10^3 Cop/ ml	Positive	Positive	Negative	Positive	Positive	70	55
43	73 X 10^3 Cop/ ml	Positive	Positive	Negative	Positive	Positive	90	85
44	400 Cop/ ml	Positive	Positive	Positive	Negative	Positive	30	35
45	38 X 10^3 Cop/ ml	Positive	Positive	Negative	Positive	Positive	46	50
46	277 Cop/ ml	Positive	Positive	Positive	Negative	Positive	29	27
47	7 X 10^3 Cop/ ml	Positive	Positive	Positive	Negative	Positive	80	65
48	800 Cop/ ml	Positive	Negative	Negative	Negative	Positive	30	25
49	38X 10^3 Cop/ ml	Positive	Positive	Positive	Negative	Positive	60	57
50	2140 Cop/ ml	Positive	Positive	Negative	Negative	Positive	12	20

PSer NO	PCR Quantitative	PCR Qualitative	HBcIgG	HBeAb	HBeAg	HBsAg	ALT	AST
51		Negative	Negative	Negative	Negative	Positive	19	25
52		Negative	Positive	Negative	Negative	Positive	23	27
53		Negative	Positive	Negative	Negative	Positive	27	32
54		Negative	Negative	Positive	Negative	Positive	19	20
55		Negative	Positive	Negative	Negative	Positive	17	18
56		Negative	Positive	Negative	Negative	Positive	50	37
57		Negative	Negative	Negative	Negative	Positive	30	32
58		Negative	Positive	Negative	Negative	Positive	36	26
59		Negative	Negative	Negative	Negative	Positive	37	24
60		Negative	Negative	Negative	Negative	Positive	22	22
61		Negative	Negative	Negative	Negative	Positive	29	30
62		Negative	Positive	Negative	Negative	Positive	35	37
63		Negative	Negative	Negative	Negative	Positive	29	27
64		Negative	Positive	Negative	Negative	Positive	28	29
65		Negative	Positive	Negative	Negative	Positive	17	18
66		Negative	Positive	Negative	Negative	Positive	25	26
67		Negative	Negative	Negative	Negative	Positive	23	20
68		Negative	Positive	Negative	Negative	Positive	20	18
69		Negative	Positive	Negative	Negative	Positive	51	35
70		Negative	Negative	Negative	Negative	Positive	19	16

The Correlation between hepatitis Be antigen with Alanine Transaminase and Aspartate transaminase

	HBeAg	
Variables	Correlation Coefficient	P. Value
ALT	0.534**	0.000
AST	0.544**	0.000
P. Valu		<0.01.

As shown in table (3) there is a highly significant correlation between hepatitis Be antigen (HBeAg) with Alanine Transaminase (ALT) and Aspartate transaminase (AST).

The Correlation between hepatitis Be antigen with Hepatitis Be antibody and hepatitis B surface antigen

	HBe-Ag	
Variables	Correlation Coefficient	P. Value
HBe-Ab	-0.400**	0.001
HBs-Ag	0.235	0.05
P. Value		<0.01

As shown in table (4) there is a highly significant statisticall inverse correlation between hepatitis Be antigen (HBe-Ag) with hepatitis Be antibody (HBe-Ab).

There is also shown in table (6) significant correlation between hepatitis B surface antigen (HBs-Ag) with hepatitis Be antigen (HBe-Ag). (P. Value <0.05).

The Correlation between hepatitis Be antibody with Alanine Transaminase and Aspartate transaminase

	HBeAb	
Variables	Correlation Coefficient	P. Value
ALT	- 0.219	0.068
AST	- 0.215	0.075
P. Value		>0.05

As shown in table (5) there is no significant statistically correlation between hepatitis Be antibody (HBeAb) with ALT and AST.

The Correlation between HBV-DNA PCR with Alanine Transaminase and Aspartate transaminase

	HBV DNA - PCR	
Variables	Correlation Coefficient	P. Value
ALT	0.565**	0.000
AST	0.451**	0.001

As shown in table (6) there was highly significant correlation between ALT with HBV-DNA PCR. (P. Value <0.01).

Also there was highly significant correlation between AST with HBV-DNA PCR. (P. Value <0.05).

The Correlation between HBV-DNA PCR with Hepatitis Be antigen (HBe-Ag) and hepatitis Be antibody (HBe-Ab)

	HBV - PCR	
Variables	Correlation Coefficient	P. Value
HBe-Ag	0.579**	0.000
HBe-Ab	- 0.365**	0.009

As shown in table (7) there was highly significant correlation between hepatitis Be antigen (HBe-Ag) with HBV-DNA PCR. (P. Value <0.01).

Also highly significant inverse correlation between hepatitis antibody (HBe-Ab) with HBV-DNA PCR. (P. Value <0.05).

** : Highly significant.

The Correlation between HBV-DNA PCR with Hepatitis B surface antigen and hepatitis B core antibody

	HBV DNA - PCR	
Variables	Correlation Coefficient	P. Value
HBsAg	0.000	-
HBcIgG	0.130	0.368

As shown in table (8) there is no correlation between hepatitis B surface antigen (HBsAg) with HBV-DNA PCR.

Also no correlation between hepatitis B core antibody (HBCAb) with HBV-DNA PCR. . (P. Value >0.05).

The comparison of normal and high Alanine Transaminase with hepatitis B surface antigen and hepatitis B core antibody

	Liver enzyme ALT (Alanine Transaminase)									
Variables	Normal ALT				High ALT				F. test	P. value
HBsAg	Mean	Std. Error	Min.	Max	Mean	Std. Error	Min.	Max	1.891	0.174
	28.6250	4.1272	17.00	51.00	61.5645	8.5350	7.0	290.0		
HBcIgG	30.3571	4.6759	17.00	85.00	64. 6607	9.3104	7.0	290.0	3.307	0.073

In table (9) the comparison of HBSAg in the patients with normal and high ALT showed no statistically significant difference in the HBsAg between two groups; P. value >0.05.

Also the table showed no statistically significant difference in the HBcIgG between two group; P. value >0.05.

The comparison of normal and high Aspartate transaminase with hepatitis B surface antigen and hepatitis B core antibody

	Liver enzyme AST									
Variables	Normal AST				High AST				F. test	P. value
HBsAg	Mean	Std. Error	Min.	Max	Mean	Std. Error	Min.	Max	1.703	0.196
	25.875	2.6822	18.00	37.00	62.1613	9.9203	7.00	405		
HBcIgG	28.8571	3.8371	16.00	65.00	65.3036	10.8767	7.00	405	2.756	0.101

In table (10) the comparison of HBSAg in the patients with normal and high AST showed no statistically significant difference in the HBsAg between two groups; P. value >0.05.

Also the table showed no statistically significant difference in the HBcIgG between two groups; P. value >0.05.

The comparison of normal and high Aspartate transaminase with hepatitis Be antigen and hepatitis Be antibody

	Liver enzyme AST									
Variables	Normal AST				High AST				F. test	P. value
HBeAg	Mean	Std. Error	Min.	Max	Mean	Std. Error	Min.	Max	28.564	0.000
	31.7413	3.6268	7.00	180.00	119.3810	23.8056	19.00	405.00		
HBeAb	67.6863	11.8690	7.00	405	32.0526	3.8711	14.0	65.00	3.280	0.075

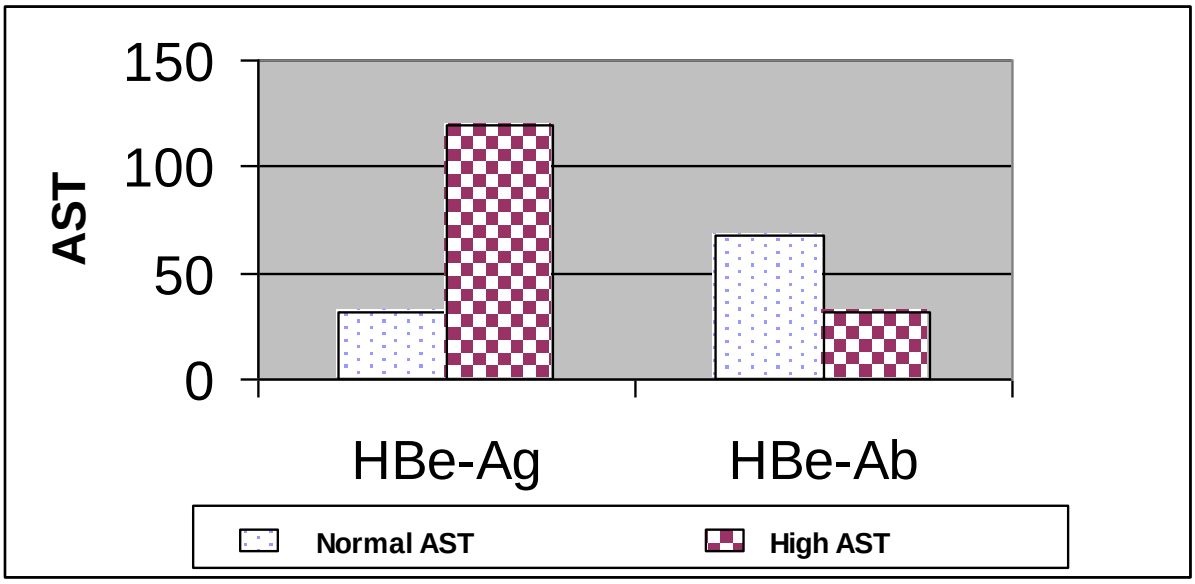

Fig. (26) : The comparison of normal and high AST with (HBeAg) and (HBe-Ab)

As shown in table (11) the comparison of HBeAg in the patient's with normal and high AST showed no statistically significant in the HBeAg between two groups P. value <0.01.

While HBeAb in the patients with normal and high AST showed no statistically significant difference in the HBeAg between two groups P. value >0.05.

The comparison of normal and high Alanine Transaminase with hepatitis Be antigen and hepatitis Be antibody

	Liver enzyme ALT (Alanine Transaminase)									
Variables	Normal ALT				High ALT				F. test	P. value
HBeAg	Mean	Std. Error	Min.	Max	Mean	Std. Error	Min.	Max	27.114	0.000
	35.5306	5.1447	7.0	250.0	109.7619	18.3155	20.0	290.0		
HBeAb	66.333	10.1425	7.0	290	34.8947	4.8792	7.0	85	3.44	0.068

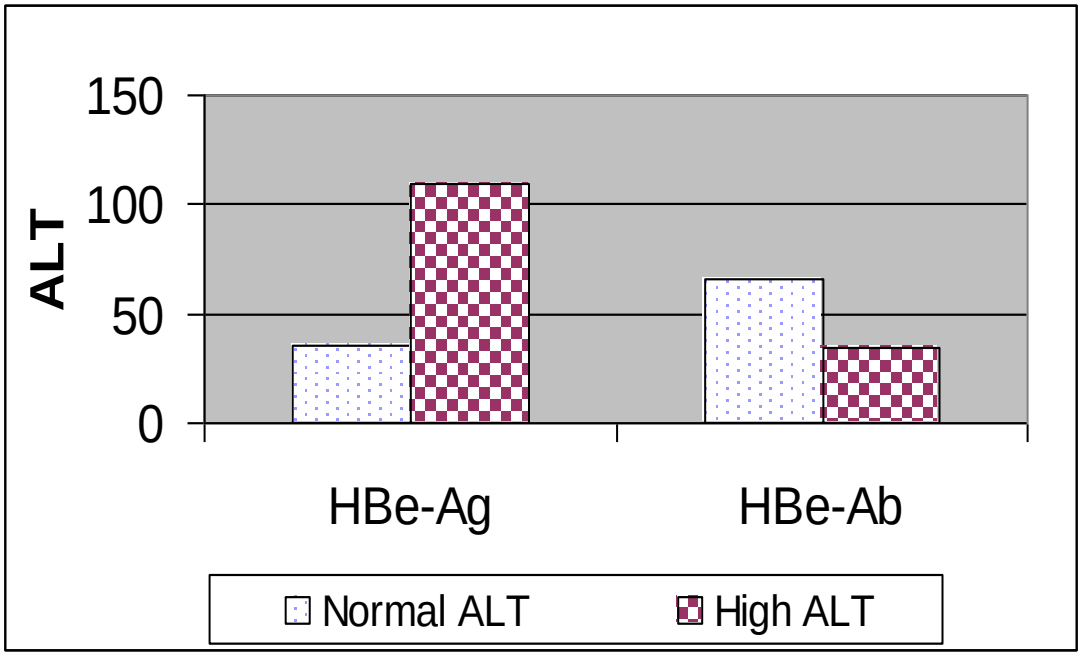

Fig. (27) :The comparison of normal and high ALT with HBe-Ag and HBe-Ab

As shown in table (12) the comparison of HBeAg in the patients with normal and high ALT showed no statistically significant difference in the HBeAg between two groups; P. value <0.01.

While HBeAb in the patients with normal and high ALT showed no statistically significant difference in the HBe-Ab between two groups; P. value >0.05.

The comparison of HBV-DNA PCR with hepatitis Be antigen hepatitis Be antibody and hepatitis B core antibody

	HBV-DNA PCR									
Variables	Negative HBV- PCR				Positive HBV-PCR				F. test	P. value
HBeAg	Mean	Std. Error	Min.	Max	Mean	Std. Error	Min.	Max	24.152	0.000
	3.8328	0.2593	2.26	7.23	5.6607	0.2502	4.00	7.4		
HBeAb	5.0273	0.2831	2.34	7.40	3.8417	0.2909	2.26	5.91	7.375	0.009
HBcIgG	3.9922	0.9162	2.31	6.57	4.6681	0.2278	2.26	7.40	0.826	0.368

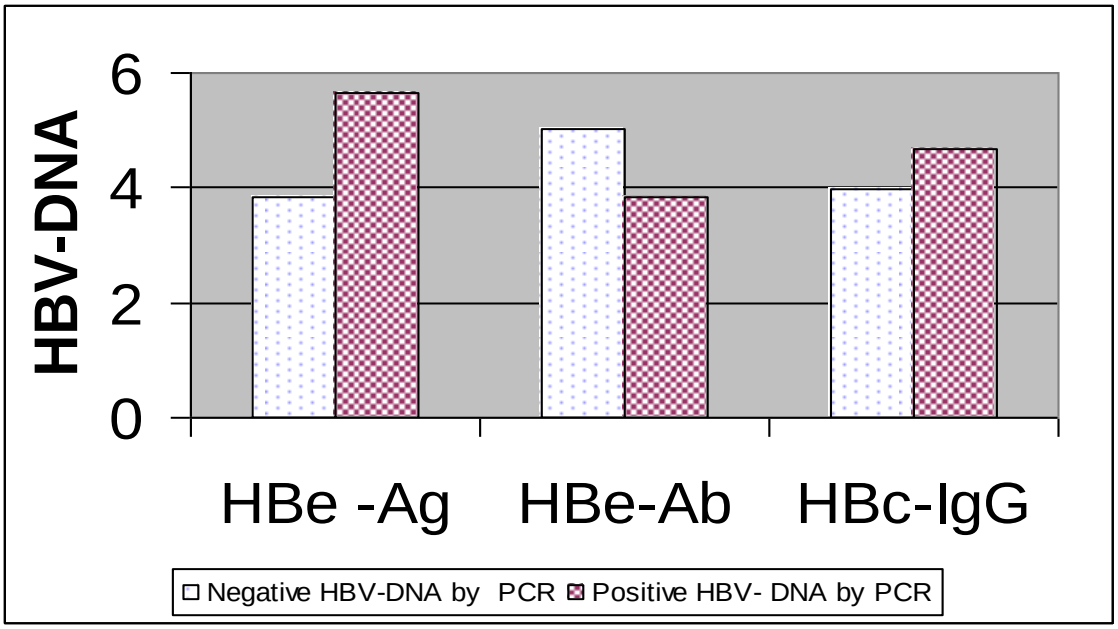

Fig (28) : The comparison of HBV-DNA by PCR with HBcIgG, HBeAb and HBeAg

The table (13) showed comparative study of hepatitis BcIgG, Hepatitis Be-Ab and hepatitis BeAg in the patients with negative and positive HBV-DNA PCR. There was highly significant difference of hepatitis Be-Ag between two groups; P. value <0.01.

There was also significant difference of hepatitis Be-Ab between two groups; P. value 0.009.

But there was no significant statistically difference for HBcIgG between tow group; P. value >0.05.

The study was conducted on 50 cases infected with hepatitis B that were selected from Cairo Lab. The age of the patients ranged from 20 to 55 years

Table (2) showed quantitative and qualitative HBV- DNA by real time PCR together with hepatitis B markers as (HBc- IgG , HBe-Ag , HBe-Ab and HBS-Ag) and liver enzymes

As shown in table (3), there is a highly significant correlation between HBe-Ag with (ALT & AST), P. <0.01.

As shown in table (4), There was a highly significant statistically inverse correlation between HBe-Ag and HBe-Ab (P0.001) , and significant correlation between HBe-Ag and HBs-Ag (p = 0.05)

In Table (5) there was no statistically significant correlation between HBe-Ab and both ALT and AST (p> 0.05)

As shown in table (6), there was highly statistical significant correlation between HBV-DNA by real time PCR and liver enzymes (ALT and AST) P.value 0.000 and <0.01 respectively.

In table (7), there was a statistically significant correlation between HBV- DNA by real time PCR and HBe-Ag (p value 0.000) and there was a statistically significant inverse correlation between HBV-DNA and HBs-Ab (P0.009)

In table (8) , there was no correlation between HBs-Ag and HBV-DNA by PCR and also no correlation HBc- IgG and HBV-DNA.

Table (9) showed a comparison of both HBs-Ag and HBc-lgG in two groups (patients with normal and the other with high ALT) . In our study there was no statistically significant difference between the two groups.

Table (10) showed a comparison of both HBs-Ag and HBc-IgG in two groups (patients with normal and the other with high AST) There was no statistically significant difference between the two groups.

Table (11) showed a comparison of HBe-Ag in two groups (patients with normal and other with high ALT). There was statistically significant difference between the two groups p. value (0.000)

HBe-Ab in two groups (patients with normal and other with high ALT). There was no statistically significant difference between the two groups

Table (12) showed a comparison of HBe-Ag in two groups (patients with normal and the other with high AST). There was statistically significant difference between the two groups.

HBe-Ab in two groups (patients with normal and the other with high AST). There was no statistically significant difference between the two groups

In table (13) there was a comparative study of HBe-Ag , HBe-Ab and HBc-IgG in patients with negative and positive HBV-DNA by real time PCR. There was highly significant difference of HBe-Ag and HBe-Ab between the two groups; P value 0.000 and 0.009 respectively.

But there was no significantly difference for HBc-IgG between the two groups

The table (14) : The genotyping of hepatitis (B) virus for 25 patients

Serial No.	Patient No.	HBsAg	HBeAg	HBV-DNA (log PCRcop/ml)	HBV Genotype
1	P_1	+ve	-ve	3.5	D
2	P_2	+ve	+ve	6.2	D
3	P_3	+ve	+ve	6.6	D
4	P_4	+ve	-ve	6.17	D
5	P_5	+ve	+ve	6.25	D
6	P_6	+ve	-ve	6.12	D
7	P_7	+ve	+ve	4.48	D
8	P_8	+ve	+ve	3.17	D
9	P_9	+ve	+ve	6.3	D
10	P_{10}	+ve	+ve	6.16	D
11	P_{11}	+ve	+ve	7.2	D
12	P_{12}	+ve	+ve	3.32	E,D
13	P_{13}	+ve	+ve	6.4	D
14	P_{14}	+ve	+ve	3.73	D
15	P_{15}	+ve	-ve	3.7	D
16	P_{16}	+ve	-ve	4.69	D
17	P_{17}	+ve	+ve	3.115	D
18	P_{18}	+ve	-ve	3.27	D
19	P_{19}	+ve	+ve	3.38	D
20	P_{20}	+ve	+ve	4.00	D
21	P_{21}	+ve	-ve	6.12	D
22	P_{22}	+ve	+ve	3.17	D
23	P_{23}	+ve	+ve	3.32	D
24	P_{24}	+ve	-ve	3.2	D
25	P_{25}	+ve	+ve	5.8	D

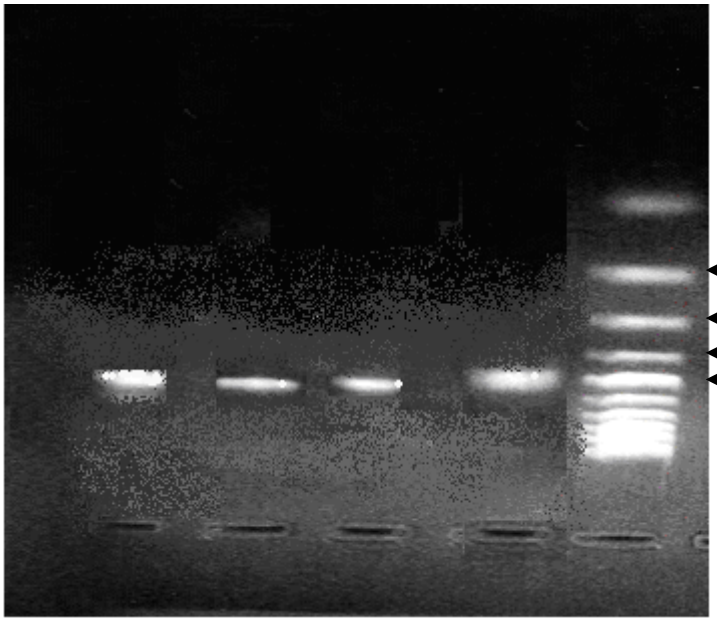

Fig. (29): Genotype D

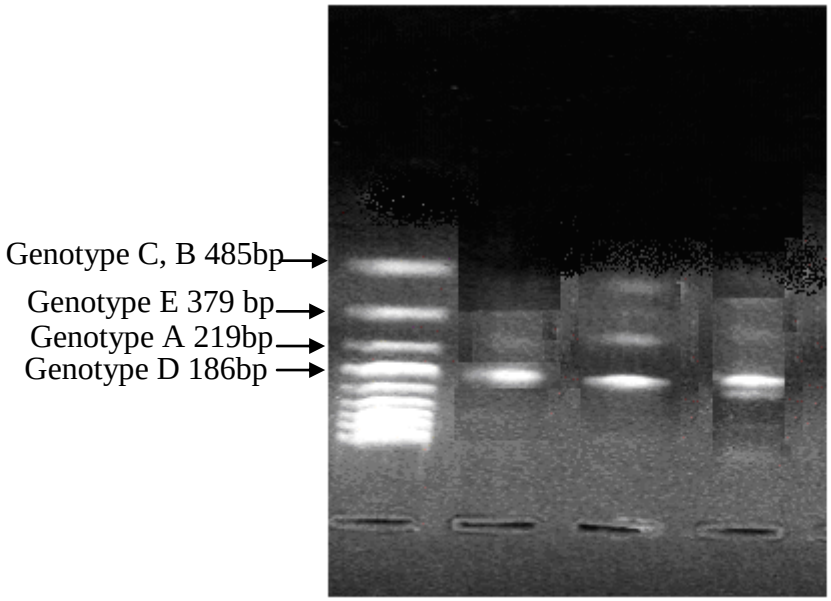

Fig. (30): Genotype D

HBV genotyping by RFLP. The seconded round PCR products which were 485bp., were digested by *AIwI* and *EarI* to fined genotype C and B. *HphI* and *NciI* digest genotype E and F. Finally genotype A and D are found by *NIaIV*.

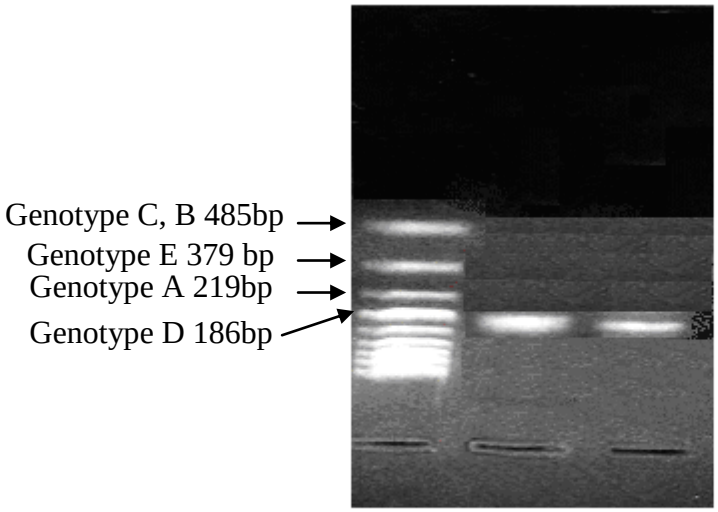

Fig. (31): Genotype D

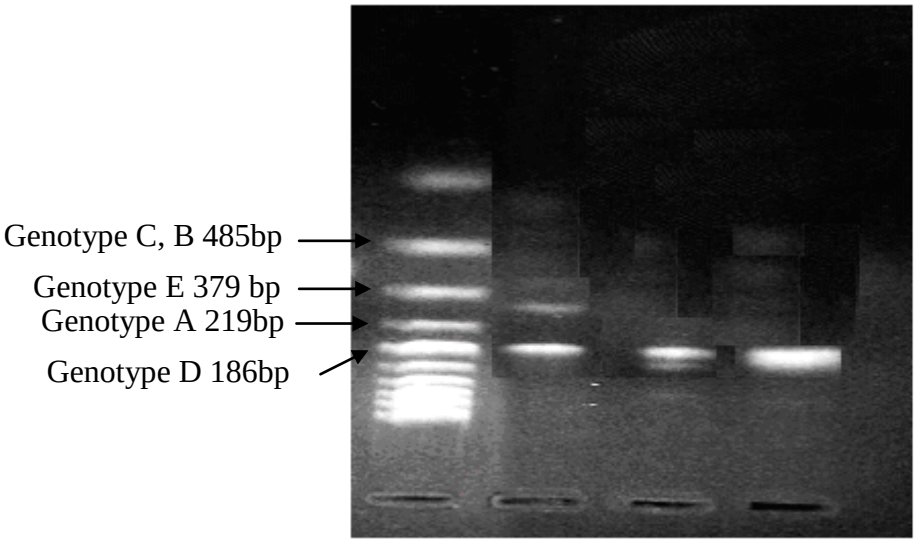

Fig. (32): Genotype D

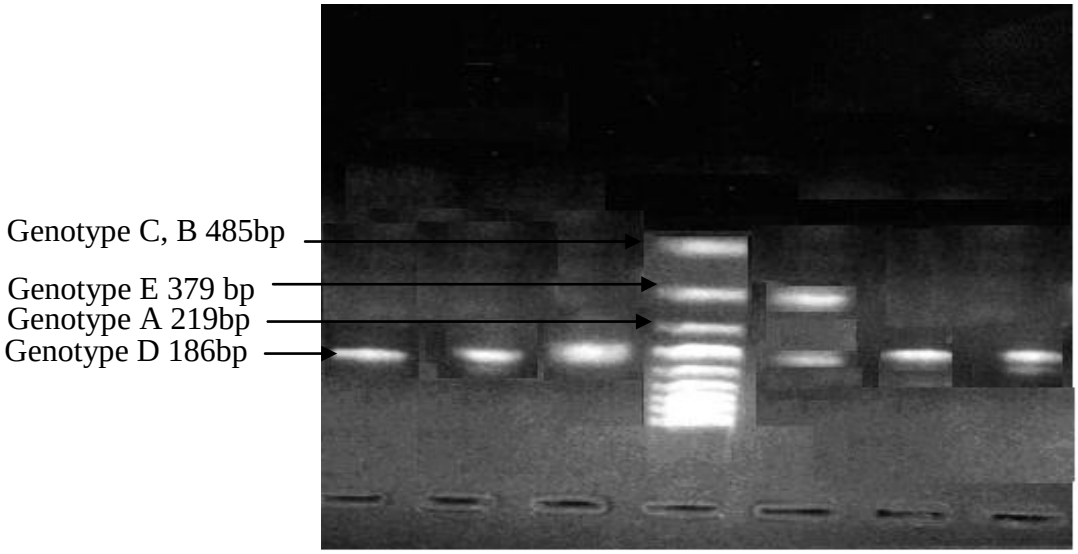

Fig. (33):Genotype D, E.

The pattern observed with these restriction enzymes as shown in Fig. (29 30, 31, 32 and 33).

In Fig. (29, 30, 31, 32 & 33) show the PCR product after digested by restriction enzyme at position 265 and 299 by NIaIV and electrophoreses on agarose gel containing ethidum bromide. The REFLP pattern was evaluated under ultra violet light.

Only genotype E had restriction site for NCIL at position 461 as shown in Fig. 33. Also we found hepatitis B genotype (D and E) in the same patient as shown in Fig. 33.

The most common genotype was D and only one case D,E.