

DNA POLYMERASE ACTIVITY: A METHOD OF DETERMINING THE INFECTIVITY OF BLOOD POSITIVE FOR anti-HBc.

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Summary

In order to determine the prevalence of continuing Hepatitis B viral replication in blood donors, the estimation of DNA polymerase activity was carried out in sera which were anti-HBc (anti-hepatitis B core antigen) positive. Of 327 samples tested 33 (10.1%) were positive. Of 71 Malay donors positive for anti-HBc, 14 (19.7%) were found to be positive for DNA polymerase activity. Of 55 Chinese donors, 11 (20%) were positive and of 38 Indian donors, 4 (10.5%) were positive for DNA polymerase activity.

INTRODUCTION

DNA polymerase is an enzyme involved in DNA synthesis, repair and replication(1). Its association with hepatitis B antigen was first demonstrated by Hirschman *et al.*(2) who described its activity in crude pellets obtained by high-speed centrifugation of three HB_sAg-positive (Australia antigen) sera. Since then the enzyme has been proven to be associated with the 27nm core of the Dane particle(3,4). It has been reported(5) that DNA polymerase-positive, anti-HBc positive (anti-core) sera are indicative of continued viral replication and therefore indicative of a highly infectious state, while sera devoid of the enzyme may contain subviral particles that are presumably non-infectious(6). The behaviour of this enzyme in the presence of high salt concentration was found to be distinctly different from that of the viral, bacterial and mammalian DNA polymerases and therefore it has been suggested that the ionic requirements of HB_sAg-associated polymerase could be used for differential assay in the presence of cellular DNA polymerases(7).

This paper reports the presence of DNA polymerase activity in the blood of some Malaysian donors which are anti-HBc positive.

MATERIALS AND METHODS

Sample collection

Blood samples from random male blood donors were collected aseptically at the Blood Services Centre, General Hospital, Kuala Lumpur. The

serum was separated from the clot within two hours after collection and frozen at -40°C. Sodium azide to a final concentration of 0.1% (w/v) was added to the samples except to the sera used for DNA polymerase assays. All assays were done within a month after collection. Slightly haemolysed and lipemic samples were not used.

Determination of anti-HBc

Detection of anti-HBc was based on the principle of competitive binding (Abbot, CorabTM) and counting of the samples was done on the Packard Autogamma Scintillation Spectrometer. Model 5110.

Determination of DNA polymerase activity

The method for detecting the enzyme was as described by Kaplan *et al.*(3). The assay measured the incorporation of mononucleotidyl residues from (³H)-deoxyribonucleoside 5'-triphosphate into acid insoluble product. Counting was done in a Packard Tricarb Model 3255 Liquid Scintillation Spectrometer.

RESULTS

Of 327 samples of blood tested for anti-HBc 164 (50.1%) were found to be positive. 33 (10.1%) were positive for DNA polymerase activity. Of these 33 samples, only 28 (84.8%) were positive for anti-HBc, indicating that not all blood positive for anti-HBc had DNA polymerase activity. Also of 71 Malay donors

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TABLE I
CORRELATION BETWEEN ANTI-HBc, HB_sAg AND DNA POLYMERASE ACTIVITY
IN MALAY BLOOD DONORS

No.	Anti-HBc	HB _s Ag	DNA Polymerase (unit)'	No.	Anti-HBc	HB _s Ag	DNA Polymerase (unit)''	No.	Anti-HBc	HB _s Ag	DNA Polymerase (unit)''
1	+	-	-	25.	+	-	-	49.	+	-	+ (2.2)
2.	+	-	-	26.	+	-	-	50.	+	-	-
3.	+	± trace	-	27.	+	-	-	51.	+	-	-
4.	+	-	-	28.	+	-	-	52.	±	-	-
5.	+	-	-	29.	+	-	-	53.	+	±	-
6.	+	-	-	30.	+	-	-	54.	+	-	-
7.	+	-	-	31.	+	-	-	55.	+	-	-
8.	+	-	-	32.	+	-	-	56.	+	-	-
9.	+	-	-	33.	+	-	-	57.	+	-	+ (1.69)
10.	+	-	-	34.	+	-	-	58.	+	-	+ (1.5)
11.	+	-	-	35.	+	-	-	59.	± trace	-	-
12.	+	-	-	36.	+	-	-	60.	+	-	+ (0.2)
13.	+	-	-	37.	+	++	-	61.	+	-	-
14.	+	-	-	38.	+	-	+ (2.16)	62.	+	-	-
15.	+	-	-	39.	+	-	+ (3.7)	63.	±	-	+ (0.24)
16.	+	-	-	40.	+	-	-	64.	+	-	+ (0.08)
17.	+	-	-	41.	++	-	+ (3.7)	65.	+	+	+ (0.02)
18.	+	-	-	42.	++	-	+ (1.0)	66.	+	-	-
19.	+	-	-	43.	+	-	-	67.	+	-	-
20.	+	-	-	44.	+	-	-	68.	+	-	+ (0.05)
21.	+	-	-	45.	+	-	-	69.	+	-	-
22.	+	-	-	46.	+	-	+ (2.0)	70.	+	-	-
23.	+	-	-	47.	+	-	+ (1.0)	71.	+	-	-
24.	+	-	-	48.	+	-	-				

TABLE II
CORRELATION BETWEEN ANTI-HBc, HB_sAg AND DNA POLYMERASE ACTIVITY
IN CHINESE BLOOD DONORS

No.	Anti-Hbc	HB _s Ag	DNA Polymerase (unit)"	No.	Anti-HBc	Hb _s Ag	DNA Polymerase (unit)"	No.	Anti-HBc	HB _s Ag	DNA Polymerase (unit)"
1.	++	-	-	19.	± trace	-	+ (3.0)	37.	+	-	-
2.	+	-	-	20.	++	-	-	38.	+	-	-
3.	+	-	-	21.	++	-	-	39.	+	-	-
4.	+	-	-	22.	++	+	-	40.	+	-	-
5.	+	-	-	23.	+	+	+ (0.08)	41.	+	-	-
6.	+	-	-	24.	+	-	-	42.	+	-	-
7.	+	-	-	25.	+	-	+ (0.05)	43.	+	-	-
8.	t	-	-	26.	+	-	-	44.	± trace	+	-
9.	+	-	-	27.	+	-	-	45.	++	-	-
10.	+	-	-	28.	+	-	-	46.	± trace	+	-
11.	+	-	-	29.	+	-	-	47.	+	-	-
12.	+	-	-	30.	+	-	+ (1.2)	48.	± trace	-	+ (0.6)
12.	+	-	-	31.	± trace	-	-	49.	+	-	-
13.	+	-	+ (4.1)	32.	+	-	+ (0.9)	50.	++	-	-
14.	+	-	-	33.	+	-	-	51.	+	-	+ (0.6)
15.	+	-	-	34.	+	-	-	52.	+	-	+ (0.6)
16.	+	-	-	35.	± trace	-	+ (0.8)	53.	+	-	-
17.	+	-	-	36.	± trace	-	-	54.	+	-	-
18.	± trace	-	+ (4.1)					55.	+	-	-

DNA POL
ACTIVITY IN BLOOD DONORS

TABLE III
CORRELATION BETWEEN ANTI-HBc, HB_sAg AND DNA POLYMERASE ACTIVITY
IN INDIAN BLOOD DONORS

No.	Anti-HBc	HB _s Ag	DNA Polymerase (unit)"	No.	Anti-HBc	HB _s Ag	DNA Polymerase (unit)*	No.	Anti- HBc	HB _s Ag	DNA Polymerase (unit)"
1.	+	-	-	14.	+	-	-	27.	+	-	-
2.	+	-	-	15.	+	-	-	28.	+	+	-
3.	+	-	-	16.	+	+	-	29.	+	-	-
4.	+	-	-	17.	+	-	+ (1.08)	30.	+	-	-
5.	+	-	-	18.	+	-	-	31.	+	-	-
6.	+	-	-	19.	+	-	+ (0.05)	32.	+	-	-
7.	+	-	-	20.	+	-	-	33.	+	-	-
8.	+	-	-	21.	± trace	-	+ (0.18)	34.	+	-	-
9.	+	-	-	22.	+	-	-	35.	+	-	-
10.	+	-	-	23.	+	+	-	36.	+	-	-
11.	+	-	-	24.	± trace	+	+ (0.71)	37.	+	-	-
12.	+	-	-	25.	+	+	-	38.	+	-	-
13.	+	-	-	26.	+	-	-				

Key: - - Negative
+ = Positive
± = Trace

* = 1 unit of DNA polymerase activity is expressed as 1 pmole residue of TMP incorporated into acid precipitable counts per 4h under the above assay conditions.

positive for anti-HBc, 14 (19.7%) were positive for DNA polymerase activity (Table I). Of 55 Chinese donors 11 (20%) were positive (Table II) and 4 out of 38 Indian donors (10.5%) were positive for DNA polymerase activity (Table III) – a significantly lower incidence than in the Malay/Chinese group. (Chi-square = 7.79, $df = 2$, $p = < 0.02$).

DISCUSSION

It has been suggested that blood with high titer of anti-HBc might be infectious(8,9) and that the presence of DNA polymerase activity in blood positive for anti-HBc is highly infectious(5). Koretz *et al.*(10) reported that post-transfusion hepatitis occurred in approximately 8% of recipients even when HB_sAg-negative (hepatitis B surface antigen) blood was given while Hoofnagle *et al.*(11) detected anti-HBc in about 1% of voluntary blood donors and 8% of

commercial blood donors. Ton *et al.*(12) has shown that about 50.1% of Malaysian donor blood is positive for anti-HBc. If 50% of donor blood is not safe for transfusion, it would be a great strain on the blood transfusion service of this country. However, it appears likely that only blood positive for both DNA polymerase and anti-HBc is highly infectious and that those without DNA polymerase activity might not be infectious at all. In our study, 28 of 327 samples investigated (8.6%) were positive for both anti-HBc and DNA polymerase activity with significantly lower activities of both parameters. in Indians compared with Malays or Chinese.

DNA polymerase activity may therefore be used for the screening of blood donors as a means of detecting the infectivity of blood to be transfused. Those found positive for DNA polymerase and anti-HBc may then be eliminated.

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